ZooKeys 525: 1–75 (2015)
doi: 10.3897/zookeys.525.6020
http://zookeys.pensoft.net

RESEARCH ARTICLE

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Taxonomic review and phylogenetic analysis of fifteen North American *Entomobrya* (Collembola, Entomobryidae), including four new species

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Academic editor: *L. Deharveng* | Received 17 April 2015 | Accepted 8 September 2015 | Published 5 October 2015

http://zoobank.org/C3C8C71F-27C6-4DCF-87A3-27C6DD6A949D

Citation: Katz AD, Giordano R, Soto-Adames F (2015) Taxonomic review and phylogenetic analysis of fifteen North American *Entomobrya* (Collembola, Entomobryidae), including four new species. ZooKeys 525: 1–75. doi: 10.3897/zookeys.525.6020

Abstract

The chaetotaxy of 15 species of eastern North American *Entomobrya* is redescribed in order to determine potential characters for the diagnosis of cryptic lineages and evaluate the diagnostic and phylogenetic utility of chaetotaxy. As a result, four new species (*E. citrensis* Katz & Soto-Adames, **sp. n.**, *E. jubata* Katz & Soto-Adames, **sp. n.**, *E. neotenica* Katz & Soto-Adames, **sp. n.** and *E. unifasciata* Katz & Soto-Adames, **sp. n.**) are described, and new diagnoses are provided for *E. assuta* Folsom, *E. atrocincta* Schött, *E. decemfasciata* (Packard), *E. ligata* Folsom, *E. multifasciata* (Tullberg), and *E. quadrilineata* (Bueker). Furthermore, previously undocumented levels of intraspecific variation in macrosetal pattern are reported, tempering the exclusive use of chaetotaxy for species delimitation. Phylogenetic relationships, estimated using both morphological and molecular data, indicate that *Entomobrya* is likely paraphyletic. The phylogenies also suggest that unreliable character homology, likely fostered by *Entomobrya*'s profusion of macrosetae, may limit the phylogenetic utility of chaetotaxy in groups characterized by an abundance of dorsal macrosetae.

Keywords

Chaetotaxy, cryptic species, phylogeny, species diagnosis, *Willowsia*

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Introduction

Studies concerning species delimitation and taxonomy of Collembola have traditionally relied on comparative morphology. For some groups, however, uncertain homology, intraspecific variation, and characters of difficult observation have limited the utility of morphological characters as tools for species diagnosis and phylogenetic inference. Recent advances in DNA sequencing technology have provided scientists with additional ways to delimit groups that lack informative morphology, such as cryptic species complexes (Frati et al. 1994, 1995, 2000; Carapelli et al. 1995, 2005; Simonsen et al. 1999; Soto-Adames 2002; Burkhardt and Filser 2005; Cicconardi et al. 2010, 2013; Felderhoff et al. 2010; Porco et al. 2012; Zhang et al. 2014c; Katz et al. 2015), but in practice most species diagnosis remains dependent on fixed characters, only obtained by rigorous morphological examination (Will and Rubinoff 2004).

Taxonomy and species delimitation of the genus *Entomobrya* Rondani, 1861 has been especially problematic due to intraspecific morphological variation and a general lack of informative taxonomic characters (Christiansen 1958b; South 1961). Conspicuous dorsal color patterns exhibited by members of this genus originally served as a practical means for species diagnosis (Bonet 1934). Other characters such as claw structure, antennal sense organs, setae types, and chaetotaxy of the male genital plate were introduced in Christiansen's (1958b) revision of North American *Entomobrya*, who attributed many color forms to intraspecific variants. These new characters marginalized color pattern as an exclusive diagnostic tool, but some are difficult to observe. Szeptycki's (1979) publication on the phylogenetic significance of dorsal chaetotaxy, introduced standard nomenclature for dorsal setae, providing a practical system to assess element homology between species. This system established chaetotaxy as the most important tool for species diagnosis in the family Entomobryidae and in theory, provided many additional characters to infer phylogenetic relationships (Soto-Adames 2008). Christiansen and Bellinger (1980, 1998) later combined traditional morphology with chaetotaxy in order to clarify species boundaries. Jordana and Baquero (2005) then simplified Szeptycki's (1979) system of body chaetotaxy and incorporated additional nomenclature for elements on the head later formalized by Soto-Adames (2008). Following this new nomenclature system, Jordana (2012) was able to delimit approximately 270 Palearctic species in subfamily Capbryinae and tribe Entomobryini using chaetotaxy.

Chaetotaxy has surely proven to be a valuable tool for springtail taxonomists, but not without complications. For some groups, especially those characterized by large numbers of setae, such as *Entomobrya*, the homology of macrosetae is not always clear. Intraspecific variation, apparent differences in setae arrangements, and differences of setae types make it difficult to determine homology between species (Soto-Adames 2008). Despite these complications, chaetotaxy still provides fixed observable differences that have been widely applied to successfully delimit species boundaries (Christiansen and Bellinger 1998; Carapelli et al. 2001; Jordana and Baquero 2005; Soto-Adames 2010; Jordana 2012). However, its utility as diagnostic and phylogenetic

characters is clearly dependent on a detailed assessment of intraspecific variation and homology between species of interest.

Christiansen and Bellinger's (1998) "The Collembola of North America" is the current authority for Nearctic Collembola species identification. However, some of their descriptions of *Entomobrya* express the need for re-examination due to a lack of informative characters for clear delineation of species boundaries, and high levels of observed intraspecific chaetotaxy and color pattern variation that suggest the presence of cryptic species complexes. In addition, their descriptions of chaetotaxy are limited and often unclear. Therefore, the primary goal of this study is to examine and document *Entomobrya* chaetotaxy to provide detailed descriptions and figures in order to clarify species boundaries and to simplify the diagnosis of eastern North American *Entomobrya* species. A total of 15 species of North American *Entomobrya* that occur east of the Mississippi River are examined and described with special emphasis on chaetotaxy and color pattern, including three cryptic species lineages identified by Katz et al. (2015) and two new species. Additionally, phylogenies, incorporating both the morphology described in this study and molecular COI sequences from Katz et al. (2015), are presented in order to explore how chaetotaxy and other morphological characters affect phylogenetic estimation.

Methods

Specimen collection and preparation

Approximately 146 specimens, representing 15 *Entomobrya* species (11 previously reported, 4 new), were examined in detail throughout the course of this study. Historical collections of *Entomobrya* from the Illinois Natural History Survey were also examined, but were not useful for the present study. Specimens preserved in 70% EtOH are old (e.g., collected prior to 1980) and in poor condition; although color pattern is often preserved, chaetotaxy and other small, but diagnostic, characters are extremely difficult to observe with confidence. Therefore, more recent material was needed to study details of chaetotaxy and other characters. Most specimens examined were collected by the senior author between 2011 and 2012 or otherwise provided by colleagues from localities throughout the USA, east of the Mississippi River. In the Material Examined sections it is assumed that the senior author collected all material, unless otherwise noted. Specimens were usually collected from leaf litter and extracted using a Berlese funnel or hand collected from bark and vegetation with an aspirator. Table 1 lists all *Entomobrya* species reported from North American.

Individuals sampled were sorted under a dissecting microscope to morphospecies according to color pattern and photographed to record dorsal thoracic and abdominal color patterns prior to slide mounting. All specimens were cleared with Nesbitt's solution and mounted on Hoyer's medium (Mari Mutt 1979) in preparation for light microscopy. The heads of specimens generally take longer to clear so they were dissected and mounted

Table 1. List of all *Entomobrya* species reported from North America. Of the 31 species, 15 were examined for this study and 16 were not included.

Species examined and described for this study	Species not included in study	
	eastern species[1]	western species[2]
E. assuta	*E. comparata*	*E. arnaudi*
E. atrocincta	*E. confusa*	*E. arula*
E. bicolor	*E. gisini*	*E. erratica*
E. citrensis sp. n.	*E. griseoolivata*	*E. kincaidi*
E. clitellaria	*E. sinelloides*	*E. nigriceps*
E. decemfasciata		*E. suzannae*
E. intermedia		*E. triangularis*
E. jubata sp. n.		*E. troglodytes*
E. ligata		*E. troglophila*
E. multifasciata		*E. washingtonia*
E. neotenica sp. n.		*E. zona*
E. nivalis		
E. quadrilineata		
E. unifasciata sp. n.		
E. unostrigata		

[1]Species reported east of the Mississippi River that were not obtained for this study

[2]Species with western North American distributions that have not been reported east of the Mississippi River. These species were not obtained for this study.

separately. Heads and bodies were both mounted dorsal-side up; the optimal position for observing important morphological characters. All illustrations were hand-drawn under a camera lucida then scanned and digitized with Adobe Illustrator CS6 software. Previously described species included in this study were identified based on morphological characters as delimited in Collembola of North America (Christiansen and Bellinger 1998).

All type specimens of *E. assuta*, *E. decemfasciata*, *E. ligata*, and *E. quadrilineata* deposited at the Illinois Natural History Survey, Champaign, IL, were examined. Types were either unavailable or their repository was unknown for most other species included in this study: *E. atrocincta*, unknown; *E. bicolor* Guthrie, unknown; *E. clitellaria* Guthrie, type stored at the Department of Animal Biology at the University of Minnesota (unavailable); *E. intermedia* Brook, unknown; *E. multifasciata*, unknown; *E. nivalis* (Linnaeus), unknown; and *E. unostrigata* Stach, type stored at the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Krakow, Poland (not examined). Additional types for *E. decemfasciata* are stored at the Museum of Comparative Zoology, Harvard (unavailable), and *E. ligata* and *E. assuta* at the American Museum of Natural History, New York (unavailable).

Four new species are described and named based on morphological and molecular differentiation: *Entomobrya unifasciata* sp. n., *Entomobrya citrensis* sp. n., *Entomobrya neotenica* sp. n. and *Entomobrya jubata* sp. n., originally identified as *Entomobrya ligata*

color form B, *Entomobrya assuta* color form D, *Entomobrya* sp. n. 1 and *Entomobrya* sp. n. 2 in Katz et al. (2015). The cryptic lineages of *Entomobrya quadrilineata* color forms B, C, and D in Katz et al. (2015) are identified as *Entomobrya decemfasciata*.

Discussion of characters

Color pattern: *Entomobrya* is characterized by distinct, but often complex and variable, color patterns. Given this diversity of color forms, color pattern was given a secondary diagnostic role partly due to confusion in the taxonomic literature caused by presumed occurrences of intra- and inter-population color pattern variation (Christiansen 1958b; South 1961). However, recent molecular phylogenetic studies have supported the resurgence of color pattern as a valid tool for species delimitation in some springtail groups (Carapelli et al. 1995, 2005; Frati et al. 1994, 1995, 2000; Simonsen et al. 1999; Soto-Adames 2002) including *Entomobrya* (Katz et al. 2015). For some species, color pattern serves as an easily observable and valid diagnostic tool when combined with traditional characters. Therefore, this study is mainly concerned with dorsal chaetotaxy and color pattern for species descriptions and diagnosis.

Apical bulb of 4th antennal segment: The apical bulb of the 4th antennal segment is present in all species of North American *Entomobrya* (except *E. sinelloides* Christiansen, 1958b) and has a number of different forms (e.g., single lobe, bi-lobed, tri-lobed). Christiansen and Bellinger (1980, 1998) incorporate these different character states into their species descriptions and diagnostic tables. However, apical bulbs are prone to misinterpretation of character states, being absent or irregular due to antennal regrowth, and moderate intraspecific variation. Therefore, this character is described here, but not considered useful for species diagnosis.

Apical sense organ of 3rd antennal segment: This setal complex consists of two sense pegs, "guard setae", and a number of differentiated setae (Chen and Christiansen 1993). These characters are very difficult to observe and lack useful variation for *Entomobrya* species diagnosis.

Differentiated setae on ventral side of 1st antennal segment: Several small, spine-like setae occur on the ventral side of the 1st antennal segment. However, no useful variation was observed for the diagnosis of species treated here.

Eye patch setae: The number of setae in the eye patch (Fig. 1A) is important for distinguishing some species of *Entomobrya*. The number of setae vary from three to six, but five is most common. Eye patch setae nomenclature in this study follows Mari Mutt (1986).

Prelabral and labral chaetotaxy: The number of setae in the prelabral and three labral rows is 4,5,5,4, typical of Entomobryidae. However, the prelabral setae (row basal to labrum), which are usually ciliate, may appear to be smooth under low magnification for some species of *Entomobrya*.

Labral papillae: The morphology of the labral papillae (Fig. 2A–D) varies considerably among species of *Entomobrya* (Christiansen 1958b; South 1961) and is relatively easy to observe, thus, descriptions of the papillae are also included in the present study.

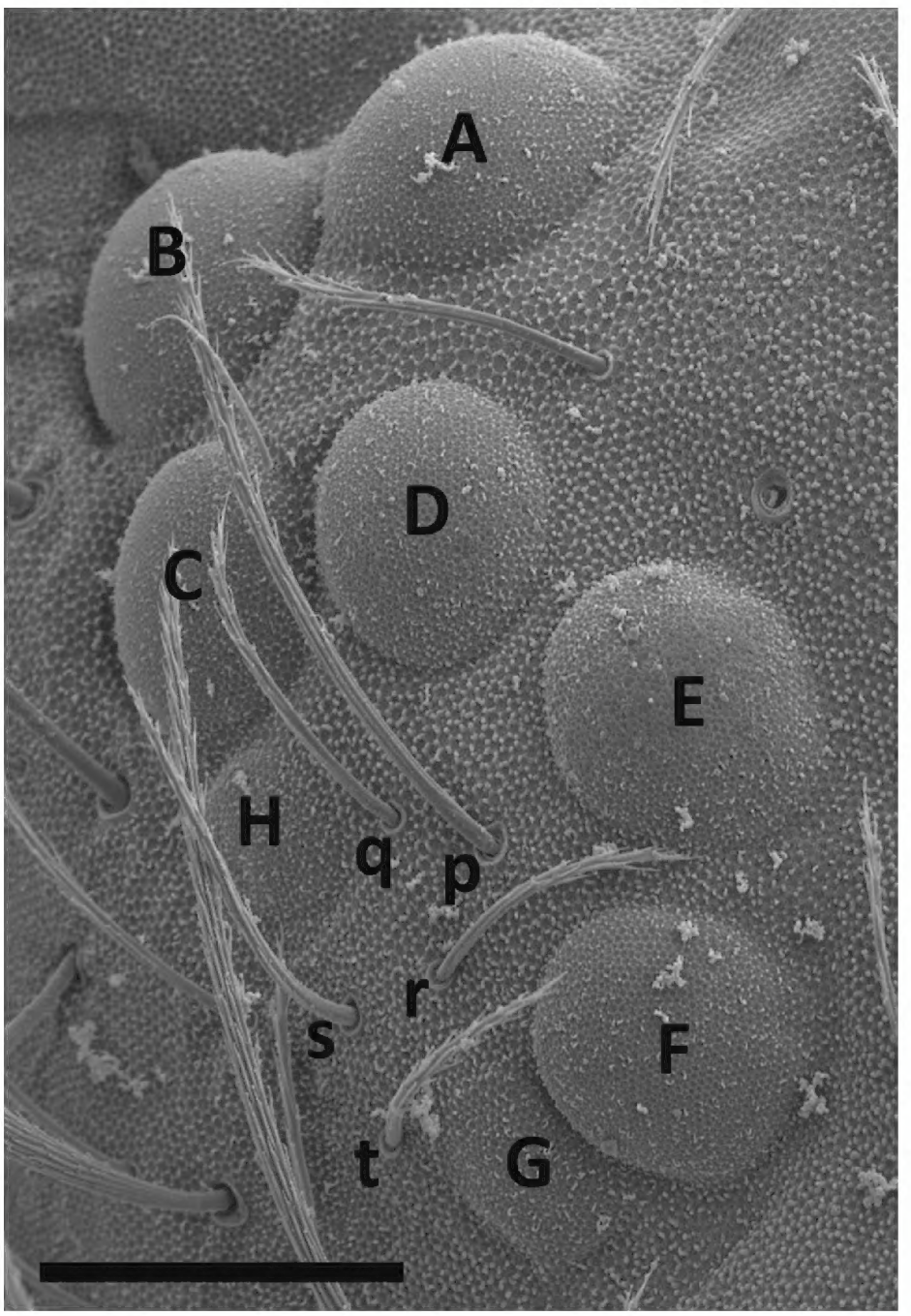
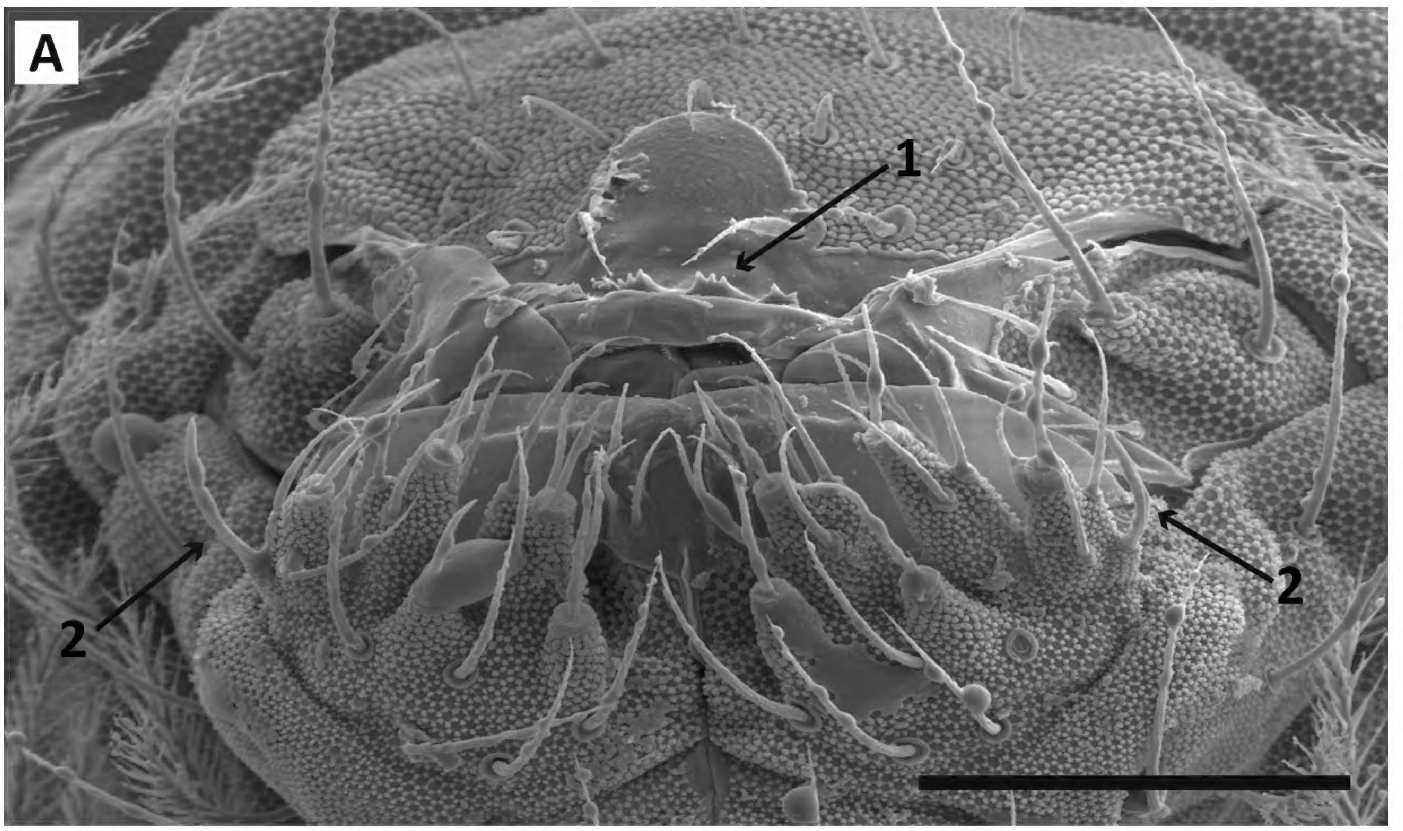


Figure 1. *Entomobrya atrocincta*. SEM photograph of right eye patch, eyes (A–H) and eye patch setae (p-t). Scale bar = 20 μm.

Labial appendage: Christiansen (1958b) incorporated the ratio of labial appendage on papilla E (Fig. 2A) to papilla length, even after recognizing the potential limitations of relative ratios (Christiansen 1954). The deformation of these soft body parts after slide mounting and pronounced variation between instars makes these measurements unreliable and not practical for diagnostic purposes (South 1961).

Labial chaetotaxy: Labial palp proximal setae, labial triangle setae, and post-labial setae have been shown to be taxonomically informative for other genera (Chen and Christiansen 1993, 1997; Soto-Adames 2010), but generally lack any useful variation in North American *Entomobrya*. For this study, all descriptions of labial chaetotaxy follow nomenclature developed by Chen and Christiansen (1993).

Dorsal chaetotaxy: The introduction of "stable" character systems for dorsal macrosetae (Szeptycki 1979; Jordana and Baquero 2005; Soto-Adames 2008) have provided a large set of characters that are relatively easy to observe and compare among different



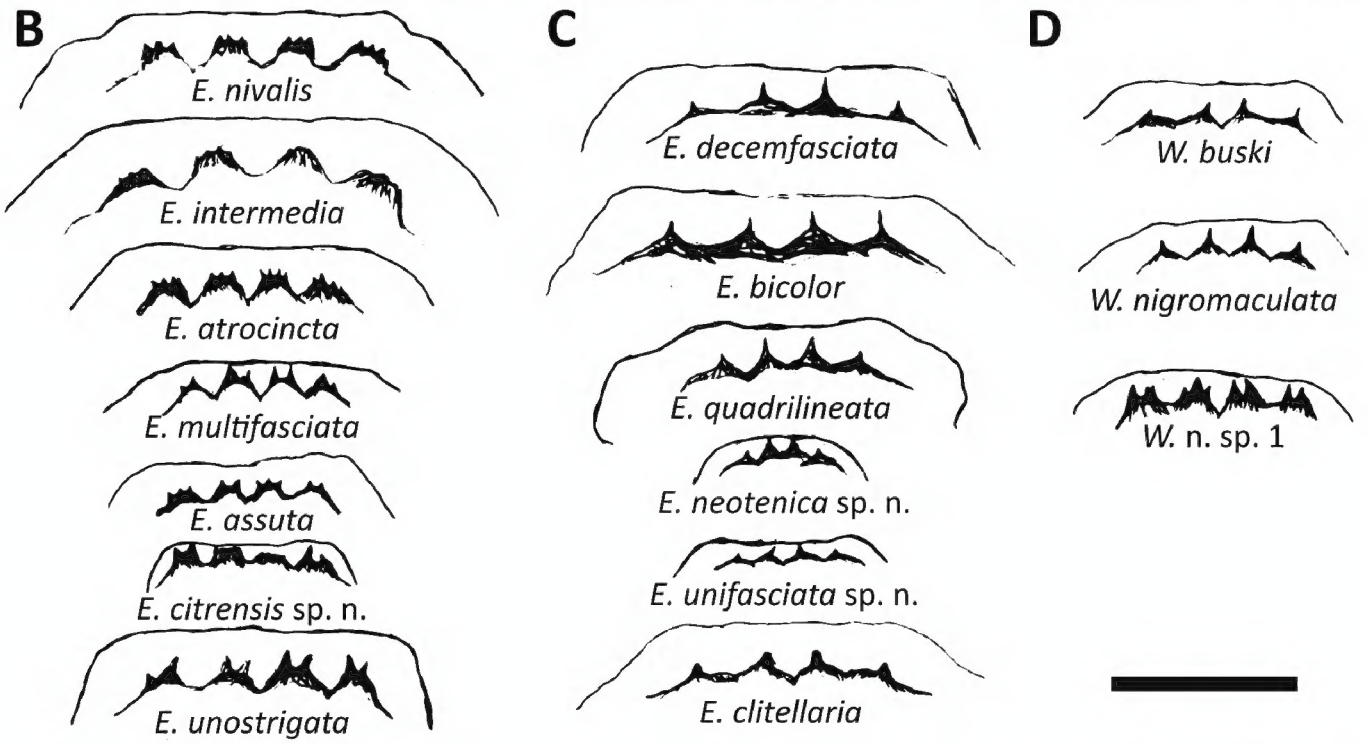


Figure 2. A *Entomobrya atrocincta*. SEM photograph of mouth cone, four labral papillae with multiple projections (1) (arrow points to 3rd papilla from left to right) and labial appendage (2) **B–D** drawings of labral papillae of *Entomobrya* and *Willowsia* species: *Entomobrya* with multiple projections per papilla (column B); *Entomobrya* with a single projection per papillae (column C); labral papillae of all *Willowsia* species occurring in the Eastern United Sates (column D). Note *Willowsia* n. sp. 1 can be differentiated by the presence of multiple projections per papilla. *E. jubata* sp. n. and *E. ligata* are not included in figure but are similar to *E. clitellaria* and *E. unifasciata* sp. n. respectively. Scale bars = 20 μm.

species. *Entomobrya* are extremely setaceous (polychaetotic), providing many diagnostic characters. However, the abundance of macrosetae and observed variation among instars (including among adult instars) makes homology assessment of each element difficult. The macrosetae inserted external to the sensilla on abdominal segments (Abd.) 1-3 and

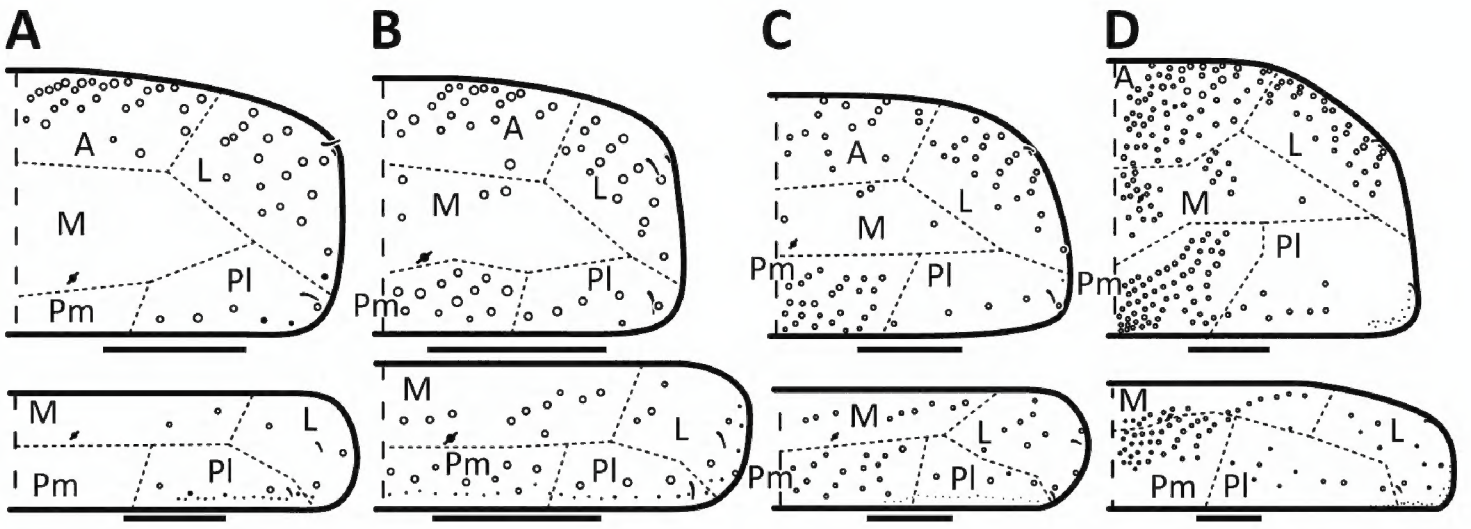


Figure 3. Differences of macrosetae abundance within thoracic chaetotaxy zones A, L, M, Pm, and Pl for Th. 2 (top) and Th. 3 (bottom): **A** *E. assuta* with the lowest number of macrosetae **B** *E. atrocincta* with a moderate number of macrosetae **C** *E. unostrigata* with a moderate number of macrosetae **D** *E. decemfasciata* with the most macrosetae. Marked differences in zone Pm for both segments clearly differentiate between some groups of *Entomobrya*. Scale bars = 100 μm.

external to the lateral bothriotricha on Abd. 4 were not included in descriptions or analysis due to extensive variation and difficulty of observation. The dorsal chaetotaxy of the head and along the dorsal median line on the mesothorax (Th. 2), metathorax (Th. 3), Abd. 1-3 and macrosetae internal to the lateral bothriotricha on Abd. 4 are easy to observe and relatively stable, thus are emphasized in the descriptions and analysis. The chaetotaxy of Abd. 5 lacks useful variation in specimens observed in this study. Thoracic zones (Fig. 3) originally described by Szeptycki (1979), differ in number of macrosetae among *Entomobrya* species and may provide some initial direction in the identification process.

Trochanteral organ: Differences in number and arrangement of small, spine-like setae on the trochanter, termed the trochanteral organ, have also been used to support the identification of some species (Christiansen 1958b). These setae are not only difficult to observe, but intraspecific variation limits their use for *Entomobrya* species delimitation (South 1961). However, the setal pattern, rather than the presences/absence or specific seta, seems to separate some species.

Male genital plate: Differences in chaetotaxy of the male genital plate can accurately delineate many North American *Entomobrya* species (Christiansen 1958a, 1958b). However, males with a well-developed plate are uncommon in the samples examined and when present, plates are difficult to observe under light microscopy, requiring electron microscopy in order to easily discern characters with certainty (Fig. 4).

Mucro and tarsal claw: Ratios of relative positions of mucronal and ungual teeth do not usually deform by the mounting process, but they depend heavily on the angle or position of the slide mount in order to properly standardize relative measurements between individuals. Additionally, these characters present few discernable differences between *Entomobrya* species and have been noted to be of little taxonomic value for European *Entomobrya* (South 1961). There are marked differences in the distance between dorsal tooth and lateral teeth on the unguis between *Entomobrya* species.

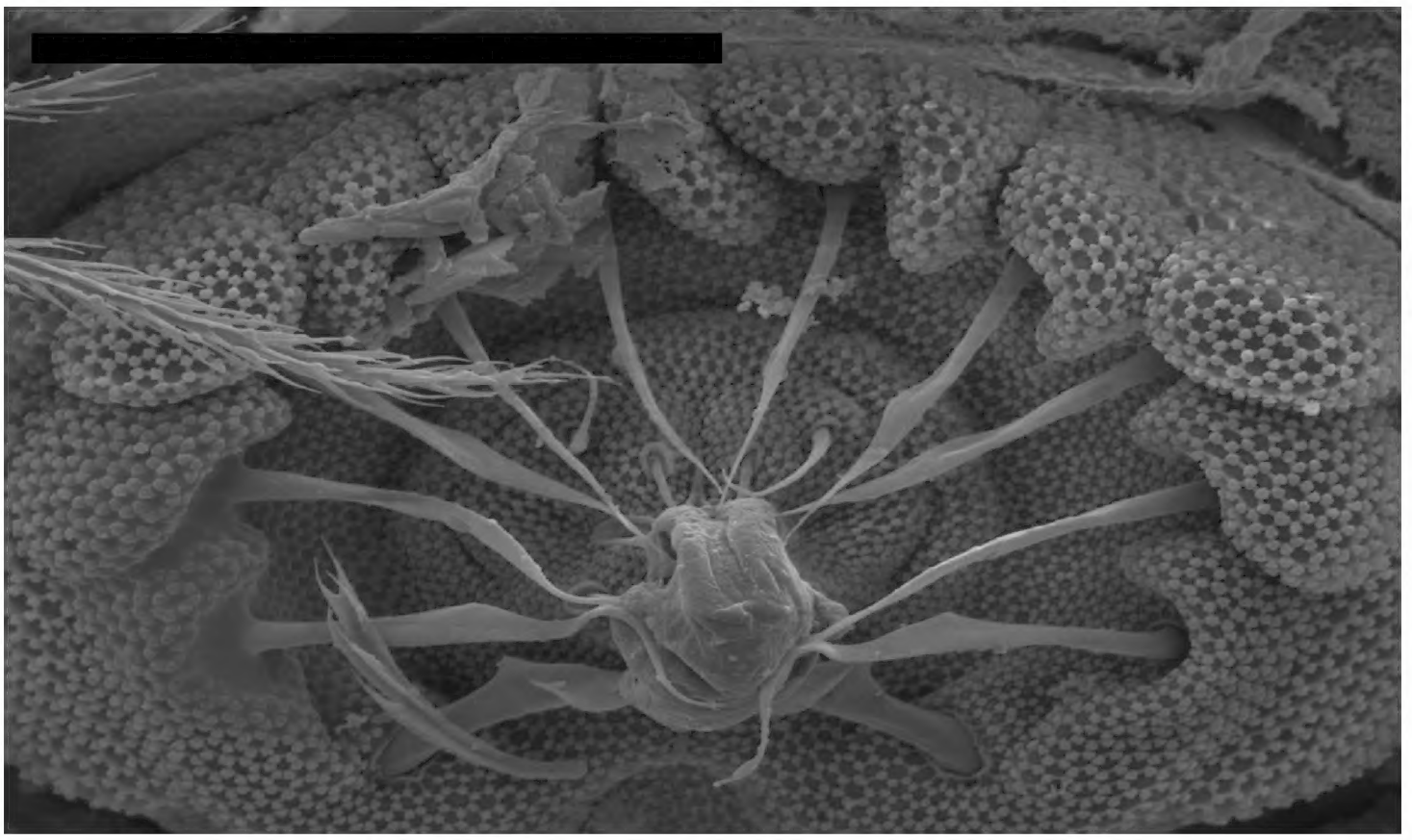


Figure 4. *Entomobrya atrocincta*. SEM photograph of papilate male genital plate. Scale bar = 20 μm.

However, these measurements are difficult to quantify due to variation and mounting inconsistencies.

Anatomical measurement ratios: Some authors have used relative anatomical length or distance ratios for *Entomobrya* species separation (Christiansen and Bellinger 1998; Jordana 2012). Although measurement ratios may provide some level of diagnostic utility, high levels of variation in ratios (Christiansen 1954, 1958b), deformation of soft tissues, and variable final position of mounted specimens reduce character consistency and reliability, thus ratio measurements are not used here as diagnostic characters.

Character nomenclature, abbreviations, and symbols

Descriptions of adult dorsal macrosetae provided in this study follow the dorsal trunk chaetotaxy nomenclature established by Szeptycki (1979) and the dorsal head chaetotaxy from Jordana and Baquero (2005) and Soto-Adames (2008). References to thoracic and abdominal segments are abbreviated as Th. (2-3) and Abd. (1-6) respectively. Symbols used in chaetotaxy descriptions are presented in Figure 5. There are seven general morphological structures, including three types of setae, recognized for the purpose of this study. Macrosetae are the primary setae, characterized by a large socket, long shaft, and are usually apically truncate or blunt (Fig. 6A). Microsetae are common type 5 (Christiansen 1958b), ciliate, short, thin, acuminate setae with very small sockets (Fig. 6A). Mesosetae are morphologically similar to microsetae, but are ostensibly longer, with larger sockets (Fig. 6A). They tend to be smaller and thinner

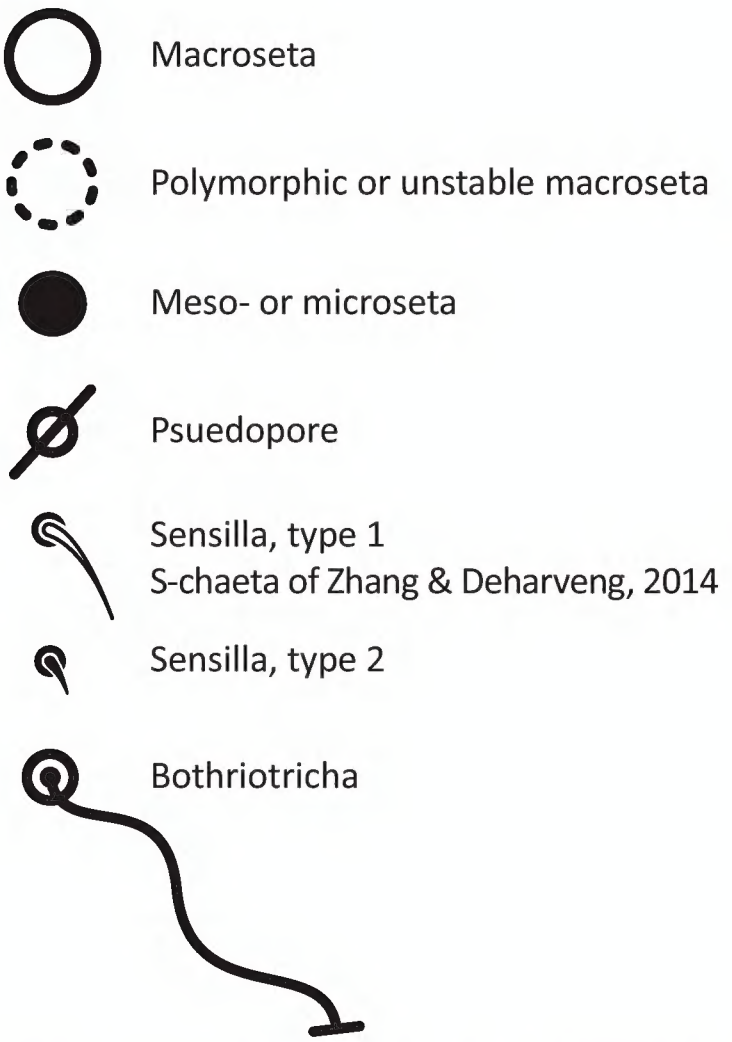


Figure 5. Symbol legend for diagrammatic figures of chaetotaxy presented in the species descriptions.

than macrosetae, but substantial variation in length and size of both setae types cause overlap. Bothriotricha (Fig. 6A) are specialized setae characterized by unique morphology; long, thin, with conspicuous ciliation. There are also two types of short, smooth, acuminate, spine-like sensilla: type 1 (S-chaeta of Zhang and Deharveng 2015) is long and more common and occur on all thoracic and abdominal segments; while type 2 (S-microchaeta of Zhang and Deharveng 2015) is short, sometimes slightly blunted, usually paired with type 1, and is only present on Th. 2, Abd. 1, and Abd. 3. Pseudopores are relatively difficult to observe and resemble sockets of macroseta, but are generally shallower and lack a thickened socket wall (Fig. 6B).

Phylogenetic analysis

In order to investigate the effects chaetotaxy and other morphological characters have on phylogenetic relationships, Bayesian and maximum likelihood phylogenetic analyses were conducted using MrBayes v. 3.2.1 (Ronquist et al. 2012) and RAxML v. 8.1.20 (Stamatakis 2014); one based only on morphological characters and an additional analysis based on combined morphology and molecular datasets.

The morphological analysis of 22 taxa, including 14 species of *Entomobrya* and 8 additional species, was based on 179 morphological characters. *Pseudosinella violenta*

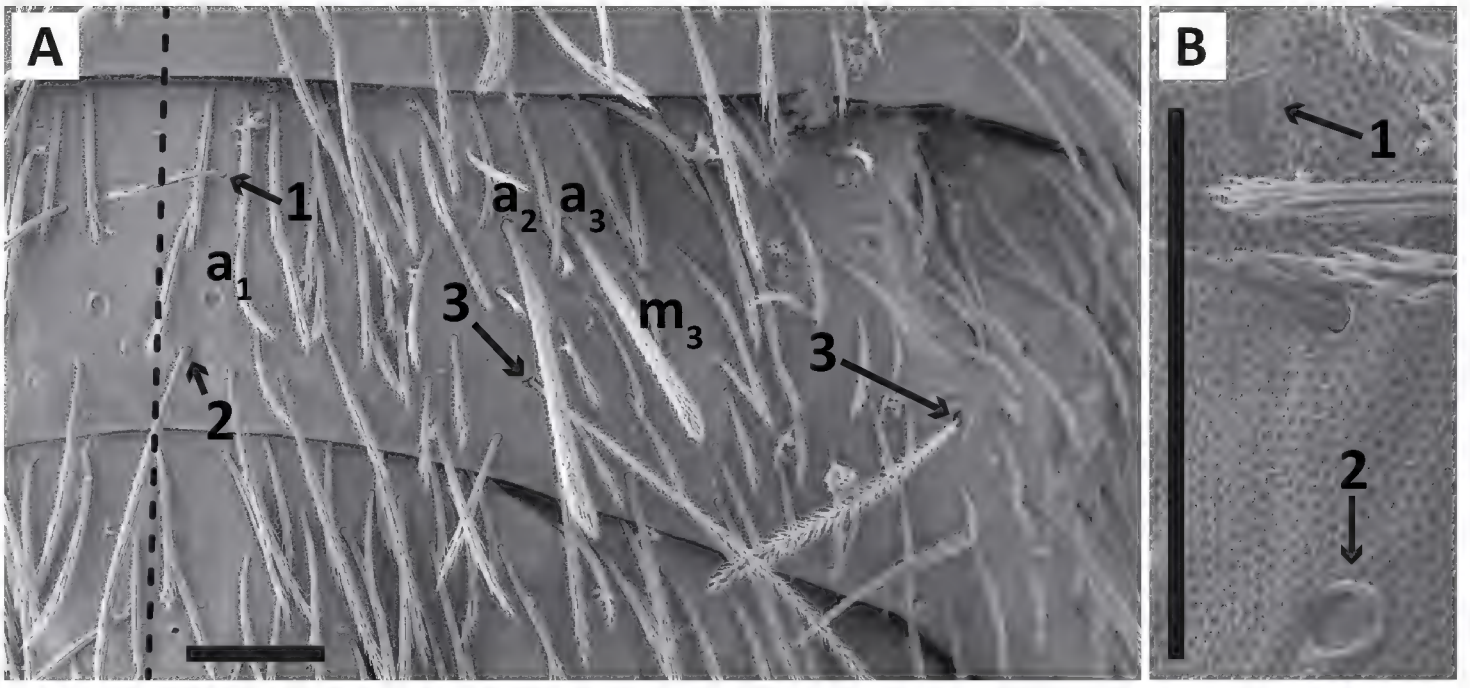


Figure 6. *Entomobrya atrocincta*. SEM photographs of selected morphological characters: **A** close-up view of 3rd abdominal segment, macroseta (socket of a_3 and m_3 are labeled), type 5 microseta (1), mesoseta (2), bothriotricha (3), the dotted line represents the medial division of Abd. 3 **B** comparison between a pseudopore (1) and macroseta socket (2). Scale bars = 20 μm.

(Folsom) was selected as the outgroup. Character state assignments (Suppl. material 1) were attained through observation of material collected for this study, except for *P. violenta* and *Seira dowlingi* (Wray), which were obtained from Soto-Adames (2010) and Soto-Adames (2008) respectively. Morphological characters were analyzed under the Mk model of evolution (Lewis 2001), with rate variation among characters (gamma distribution), four independent runs, starting from random trees, four Markov chains (temp=.5), and 25,000,000 generations, sampling every 1000th generation. Default values were used for all other parameters.

The combined analysis incorporating complete COI sequences (1539 bp) for 89 exemplars (See Katz et al. 2015 for specific details regarding specimens, GenBank accession numbers, gene choice, DNA extraction, amplification, sequencing, and primer development) with *P. violenta* as the outgroup. The appropriate model of sequence evolution (GTR+I+G), was selected using jModeltest (Posada 2008), whereas the Mk model (Lewis 2001) was implemented for the morphology dataset. Morphology was not examined for sequenced individuals due to the destructive process of DNA extraction; therefore character states were attained from other individuals of the same species or from descriptions in the literature (Soto-Adames 2008, 2010) and were added to corresponding OTU's in the matrix. Bayesian analyses were conducted using MrBayes on the CIPRES Science Gateway (Miller et al. 2010), convergence was assessed by observation of average standard deviation of split frequencies values below $p < 0.003$, a 25% burn-in was used, and all posterior probabilities and consensus trees were computed in MrBayes. The maximum likelihood analyses were conducted using RAxML's rapid bootstrap algorithm incorporating 1000 bootstrap replicates.

Character state assignments

Dorsal setae were identified by their relative positions to bothriotricha, sensilla, pseudopores, and to neighboring setae, following descriptions provided by Szeptycki (1979), Jordana and Baquero (2005), and Soto-Adames (2008). All macrosetae labeled in figures for species descriptions were included as characters in the phylogenic analysis (See Suppl. material 1 for morphological character matrix). Macrosetae external to sensillum on Abd. 1-3 and external to bothriotricha on Abd. 4 were not included in descriptions or analysis due to uncertain homology. Characters states for setae were defined as present, absent, or polymorphic (i.e., variable between individuals of the same species). Macrosetae were considered absent if meso- or microsetae were present in corresponding position. Polymorphic states are indicated in the figures as dotted circles. Other morphological characters included in the phylogenetic analysis are number of eye patch setae, labral setae (smooth or ciliate), labral papillae (smooth, single projection, or multiple projections), setae within labial triangle (ciliate or smooth), antennal bulb (absent or present), scales (absent or present), and dental spines (absent or present). See Suppl. material 1 for a complete list of all 179 morphological characters and character states circumscription used in this analysis.

Results

Species descriptions and taxonomy

Family Entomobryidae Schäffer, 1896
Entomobryini Schäffer, 1896 sensu Soto-Adames et al. 2008

***Entomobrya* Rondani, 1861**

This genus characterized by having 8+8 eyes within black or dark blue patches of pigment, a bidentate mucro with a smooth basal spine, basic chaetotaxy formed by type 5 microsetae and the absence of antennal sub-segmentation, scales, dental spines, and differentiated "smooth" setae on the inner surface of the hind tibiotarsus.

In addition, all species treated here have an apical antennal bulb; main sensilla on 3rd antennal segment sense organ thin, smooth, blunt and peg-like; differentiated smooth setae on ventral side of 1st antennal segment of two types, short and spine-like and long and seta-like; labral setae 5,5,4 and smooth; outer maxillary lobe of maxilla with subapical and apical setae smooth and subequal, and sublobal plate with three smooth seta-like appendages; lateral appendage of labial papilla E slightly curved, relatively thick, blunt; all post labial setae type 5; unguis with one outer, two lateral and four inner teeth, and a lanceolate unguiculus; and mucronal spine smooth; and most have all posterior setae of labial triangle ciliate, as M1, r, E, L1, L2, with r significantly smaller than other setae and A1-A5 smooth.

More general morphological descriptions of this genus are provided in Christiansen (1958b), Stach (1963), Christiansen and Bellinger (1998), and Jordana (2012). Chaetotaxy provided in the following descriptions follows the nomenclatural systems established by Szeptycki (1979) and Jordana and Baquero (2005) with some modifications.

Informative diagnostic adult chaetotaxy characters of all *Entomobrya* species treated here are listed in Table 2. These characters were specifically chosen for their ease of observation, stability (lack of polymorphisms), and confident homology.

Table 2. Informative diagnostic adult chaetotaxy for the separation of all *Entomobrya* species in this study[5,6]. These characters were specifically chosen for their ease of observation, stability, and lack of polymorphic states. Refer to species remarks for more specific diagnostic characters. Parentheses indicate a rarely observed state.

	Head macrosetae[1]					**Th. 2 macrosetae**[1]		**Abd. 2 macrosetae**[1]			**Abd. 3 macrosetae**[1]		**Prelabral setae**[2]	**Number of eye patch setae**[3]	**Labral papilla**[4]
Species	M_{3i}	S'_0	S_{4i}	Ps_3	Ps_5	m_2	m_5	a_2	a_3	m_{3ep}	a_1	a_2			
E. assuta	0	0	1	0	1	0	0	0	0	0	0	1	0	5	0
E. atrocincta	0(1)	0	1	0	1	1	0	1	1(0)	0(1)	1	1	1	5	0
E. bicolor	1	0	1	0	0	1	1	1	1	1	0	1	1	5	1
E. citrensis sp. n.	0	0	1	1	1	0	0	0	1	0	0	1	0	5	0
E. clitellaria	0	0	1	0	1	1	1	1	1	1	0	1	1	5	1
E. decemfasciata[5]	1	0	1	0	1	1	1	1	1	1	0	1	1	5	1
E. intermedia	0	0	0	0	1	1	1	1	1	0	1	0	1	5	0
E. jubata sp. n.	0	1	1	0	0	1	1	1	1	1	0	1	1	5	1
E. ligata[6]	0	0	1	0	1	1	0	1	0	1	0	0	0	3	1
E. multifasciata	0	0	0	0	1	1	0	1	1	0	1	1	1	5	0
E. neotenica sp. n.	0	0	0(1)	0	1	1	0	1	0	0	0	0	0	3	1
E. nivalis	0	0	1	P	1	1	1	1	1	1	1	0	1	5(6)	0
E. quadrilineata[5]	1	0	1	0	1	1	1	1	1	1	0	1	1	5(6)	1
E. unifasciata sp. n.[6]	0	0	1	0	1	1	0	1	0(1)	1	0	0	0	3	1
E. unostrigata	0	0	1	0	1	0	1	1	1	1	1	0	1	5	0

[1] Character states: absent (0), present (1), polymorphic (P).

[2] Character states: smooth or finely ciliate (0), ciliate (1).

[3] Refer to Figure 1 for SEM photograph of eye patch setae.

[4] Character states: multiple projections (0), single projection (1). Refer to Figure 2 for SEM photograph and drawings of labral papillae.

[5] *E. decemfasciata* and *E. quadrilineata* cannot be separated by these diagnostic characters. Color pattern is critical for diagnosis.

[6] *E. ligata* and *E. unifasciata* sp. n. cannot be separated by these diagnostic characters. Color pattern is critical for diagnosis.

***Entomobrya assuta* Folsom, 1924**
Figs 2, 3A, 7, 8, 39

Description. *Body shape and color pattern*. Body dorso-ventrally flattened. Dorsal color pattern highly variable, and in many cases, without clear discrete forms (Fig. 7). Patterns usually consisting of black or dark blue pigment on a white, yellow, orange, or light purple background. Thorax pigmentation variable. Sometimes Th. 2 and Abd. 3 entirely dark, forming two strong transverse bands. All forms studied have a dark transverse band across the posterior margin of Abd. 2. Abd. 3 entirely dark or with two or three pale spots. Abd. 4 and 5 also with 2-3 pale spots each. Posterior margin of Abd. 4 usually lacking pigment, forming an irregular pale transverse band. Antennae usually entirely purple, but 1st antennal segment at times considerably lighter.

Head. Apical bulb of 4th antennal segment usually bilobed, sometimes simple or, rarely, trilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Prelabral setae finely ciliated, seemingly smooth at low magnification under light microscopy. Distal margin of labral papillae with 2-3 small spine-like projections (Fig. 2). Dorsal head chaetotaxy reduced in comparison with other species (Fig. 8A); macrosetae An'_0, A_6, M_{3i}, S'_0, S_1, Pi_1, Pa_2, Pa_3, Pm_2, and Pm_{1i} always absent; An_{3a3} seen in one individual; M_3 present in 1/4 of individuals observed; S_0 and Pa_1 usually present. Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax. Thoracic chaetotaxy extremely reduced but stable, without macrosetae variation in specimens studied. A row of microsetae present along entire posterior margin of Th. 2 and Th. 3 (not displayed in figures). Th. 2, with a_5 and 5 posterior macrosetae (Fig. 8B): p_4, p_5, p_6, p_{6e}, and p_{6ep}. Th. 3, with 7 macrosetae (Fig. 8C): a_5, a_6, m_5, m_6, m_7, p_4, and p_6; macrosetae a_1, a_2, and a_3 absent. Chaetotaxy of zone Pm extremely reduced for both thoracic segments, without macrosetae (Figs 3A; 8B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 23 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy reduced but stable, no macrosetae variation observed. Abd. 1 with 1 macroseta only (Fig. 8D); row of microsetae along entire posterior margin present (not displayed in figure). Abd. 2 with 3 macrosetae: m_3, m_{3e}, and m_5 (Fig. 8E). Abd. 3 with 4 macrosetae: a_2, m_3, pm_6, and p_6. Mesosetae a_{2a}, inserted slightly anterior and exterior to a_2, sometimes with relatively large socket resembling socket of macroseta a_3, but due to mesoseta a_{2a}'s close proximity to a_2, it is most likely a duplicate of a_2 rather than homologous to a_3 (Fig. 8F). Abd. 4 with 4 inner macrosetae (Fig. 8G). Mucronal teeth subequal.

Remarks. *Entomobrya assuta* is the only species with the color pattern as described above in combination with the absence of all the following macrosetae: head Ps_3, Th. 2 m_2 and m_3, Abd. 2 a_2, a_3 and m_{3ep}, and Abd. 3 a_1 (see Table 2 for additional diagnostic characters). Among all species observed in this study, only *E. assuta* and *E. citrensis* sp. n. share this unique pattern of substantially reduced dorsal chaetotaxy. *Entomobrya assuta* and *E. citrensis* sp. n. share very similar chaetotaxy and color pattern. In fact, *E.*

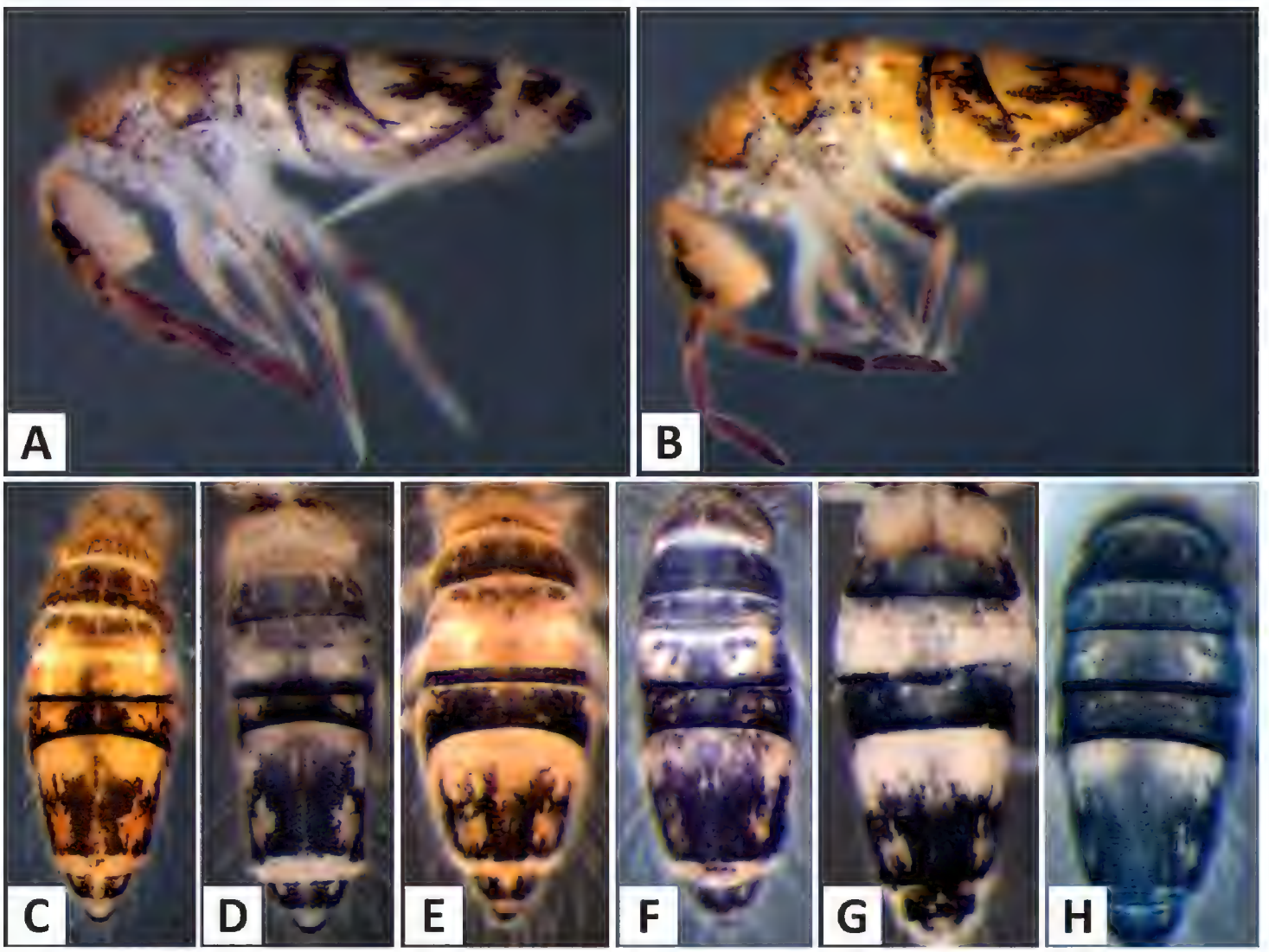


Figure 7. Color patterns of *Entomobrya assuta*. Photographed specimens are from the following localities: **A** Champaign Co., IL **B** Champaign Co., IL **C** Champaign Co., IL **D** Knox Co., TN **E** Knox Co., TN **F** Mason Co, IL **G** Knox Co., TN **H** Iroquois Co., IL.

citrensis sp. n. was at first considered to be an undescribed color form of *E. assuta*, but molecular data provides evidence for their separation (Katz et al. 2015).

Subsequent comparative morphological observations between the two forms show that head macroseta Ps_3 and Abd. 2 macroseta a_3 are both absent in *E. assuta*, but present in *E. citrensis* sp. n.; additionally, *E. citrensis* sp. n. has a complete, dark transverse band located medially across Abd. 4, whereas this band is absent in *E. assuta*. Labral morphology also separates these species, *E. assuta* has relatively uniform labral papillae, each with two to three seta or spine-like projection, whereas *E. citrensis* sp. n. has up to five minute bumps or serrations on the two internal papillae and only two larger spine-like projections on the two external papillae (Fig. 2). It should be pointed out that the last character may be variable and should be used in combination with chaetotaxy and color pattern for diagnosis.

Christiansen and Bellinger (1998) reported seven different color forms of *E. assuta*, each occurring in separate localities across North America. Many of these color forms were not sampled for this study, and in view of the discovery of *E. citrensis* sp. n. it is possible that some of them may represent distinct species. Future determinations of *E. assuta* will have to be based on analysis of chaetotaxy and other morphological characters outlined in the present description and not just color pattern.

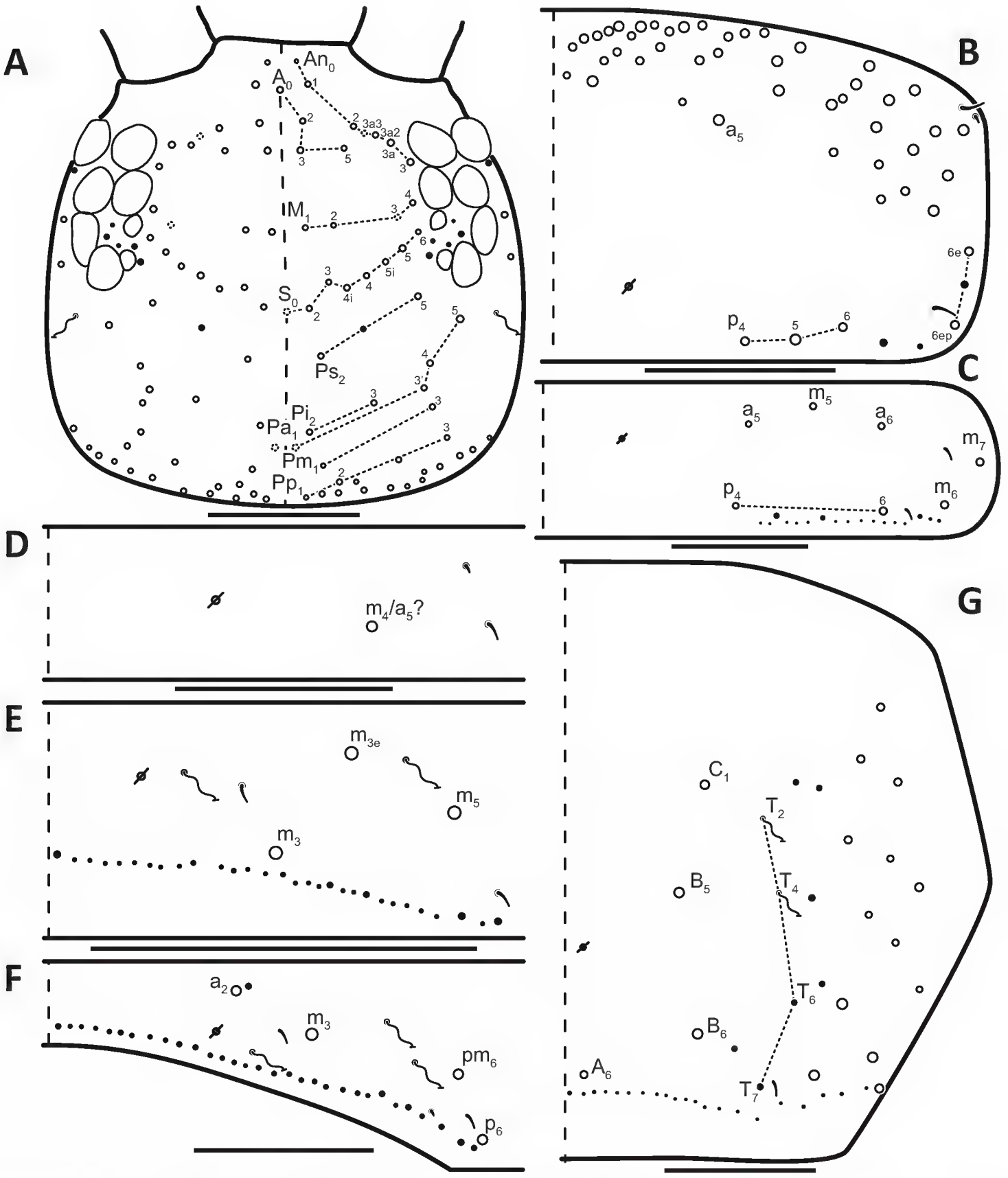


Figure 8. Dorsal chaetotaxy of *Entomobrya assuta*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

Entomobrya assuta appears to be an intrusion of a southern subtropical or tropical *Entomobrya* lineage into the Nearctic region. Christiansen and Bellinger (1998) noted that the dorsal and genital chaetotaxy of *E. assuta* is more similar to tropical rather than Nearctic species. In fact, the reduction in chaetotaxy approaches that seen in *E. longiseta* Soto-Adames and *E. linda* Soto-Adames from the Caribbean, more than other Nearctic forms.

Distribution. Endemic to North America (Suppl. material 2: A).

Material examined. USA: *Cotypes* (J. W. Folsom), 2 on slide, Vermont, Clarendon, under bark Mar-April 1898 (O. W. Barrett), INHS Cat. No. 528,332; *Cotypes* (J. W. Folsom), 5 in vial, same information as above; *Cotypes* (J. W. Folsom), 4 in vial, Geneva, N. Y., June 18, 1917, beneath apple bark (H. Glasgow). Other material examined: 2 in vial, Alabama, Covington Co., Conecuh National Forest, off Co. Rd. 11 (31.07900,-86.61203), under bark, 2.i.2012 (A. Katz & M. DuBray), AK12-6; 3 on slides, 20 in vial, Florida, Taylor Co., Econfina State Park (30.0656,-83.91066), under bark, 9.viii.2011, AK11-116; 2 in vial, Illinois, Champaign Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), in bird nest, 8.v.2011, AK11-26; 3 on slides, 5 in vial, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14462,-88.16543), on low-lying vegetation, 7.vii.2011, AK11-60; 1 on slide, Illinois, Champaign Co., Urbana, bird nest, 28.ix.1957 (R. Hurley); 5 on slides, Illinois, Iroquois Co., Iroquois County Forest Preserve (40.99279,-87.59734), under bark, 19.viii.1989 (F. Soto-Adames); 1 in vial, Illinois, Jasper Co., Sam Parr State Fish and Wildlife Area (39.03293,-88.12516), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-73; 2 in vial, Illinois, Jo Davies Co., South Blanding Rd., Stevenson Property (42.29895,-90.36967), under bark, 27.viii.2011, A11-148; 1 on slide, 6 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), under bark, 26.viii.2011, AK11-142; 1 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), beating vegetation, 26.viii.2011, AK11-143; 4 in vial, Illinois, Jo Davies Co., South River Rd., Asgard Vent 1 (42.30170,-90.40345), under bark, 27.viii.2011, AK11-153; 1 on slide, 7 in vial, Illinois, Jo Davies Co., South River Rd., Asgard Vent 2 (42.30378,-90.40108), under bark, 27.viii.2011, AK11-157; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from bird nest, 10.iv.2011, AK11-6; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from squirrel nest, 10.iv.2011, AK11-8; 1 in vial, Illinois, Mason Co, Revis Hill Nature Prairie Reserve (40.15246,-89.85330), beating vegetation, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-86; 2 on slides, 4 in vial, Illinois, Mason Co., Sand Ridge State Park (40.40892,-89.87590), beating branches, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-82; 2 on slides, 8 in vial, Illinois, Piatt Co., Lodge Park (40.06709,-88.56596), under bark, 23.vii.2011, AK11-100; 1 in vial, Illinois, Pike Co., Lincoln's New Salem State Park (39.96868,-89.83386), beating grasses, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-90;1 on slide, 3 in vial, Illinois, Pope Co., Lake Glendale (37.41350,-88.65982), under bark, 24.ix.2011, AK11-160; 1 on slide, Illinois, Vermilion Co., University of Illinois Observatory, Nixon Fork, leaf littler, 25.iv.2009 (F. Soto-Adames & L. Deem), FD09-25; 1 in vial, Illinois, Vermilion Co., Kennekuk Cover County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), aspirated from bushes, 16.vi.2011 (A. Katz & F. Soto-Adames), AK11-59; 3 on slides, Illinois, Will Co., Braidwood (41.25118,-88.19494), soil and leaf litter, 6-8.ix.2011 (F. Soto-Adames); 2 on slides, Michigan, Ingham Co., Michigan State University, Baker Wdlt. (42.66527,-84.36264) under bark of standing dead pine, 24.vii.2008 (E. C. Bernard), BW-11; 1 on slide, Michigan, St. Clair Co., Algonac State Park (42.65447,-82.52430), under

bark of recently fallen maple, 25.vii.2008 (E. C. Bernard), ASP-15; 8 in vial, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 29.vi.2012, AK12-50; 7 on slides, 30+in vial, Tennessee, Knox Co., University of Tennessee, Ag. Campus, Morgan Hall (36.01023,-83.93829), on moist fallen bark, April 2010 (E. C. Bernard).

Entomobrya atrocincta Schött, 1896

Figs 1, 2, 3B, 4, 6, 9, 10, 39

Description. *Body shape and color pattern*. Sexually dimorphic in color pattern and body shape. Males and females with variable but characteristically different color patterns (Fig. 9). Male body relatively cylindrical, slender, with bright orange background with black pigment usually forming a thick and complete transverse dorsal band covering posterior margin of Th. 2 and all of Th. 3 and Abd. 1, band sometimes absent. Male light form without dark pigment on Th. 2 through Abd. 6 (except sometimes along anterior margin of Th. 2). Male dark form with band covering Th. 2 through Abd. 2, irregular pigment patterns sometimes forming 1+1 orange spots on Abd. 3, and two narrow longitudinal stripes connected by transverse band on posterior margin of Abd. 4 (Fig. 9A–D). Females with slightly dorso-ventrally flattened body and slightly larger than males. Female color pattern strikingly different from males, white or light yellow background with black, dark blue or purple pigment forming transverse bands across the posterior margins of Th. 2 through Abd. 4. All females with two longitudinal stripes or triangular extensions connected by two transverse bands on Abd. 4; one incomplete medial band and another complete band on posterior margin of Abd 5 (Fig. 9E–I). Mesonotum white in both sexes, lacking pigment except for a small irregular band across anterior margin. Medial area of Th. 2 relatively transparent and fat bodies visible through cuticle under a dissecting microscope. Both males and females with purple pigment usually extending from apical end of 2nd antennal segment through apex of 4th antennal segment.

Head. Apical bulb of 4th antennal segment located in deep pit, usually simple, sometimes with up to four distinct lobes. Long differentiated smooth setae on ventral side of 1st antennal segment ≈2.5x short setae. Prelabral setae ciliate. Ornamentation of distal margin of labral papillae with 3-4 small seta or spine-like projections (Fig. 2). Dorsal head chaetotaxy variable (Fig. 10A); macrosetae An'_0, An_{3a2}, An_{3a3}, S'_0, S_6, Ps_3, Pi_1, Pa_3, and Pm_{1i} always absent; S_0 usually present, M_{3i} usually absent, A_6 present in roughly half of observed specimens. Eyes G and H small and subequal; eye patch with 5 setae.

Thorax. Chaetotaxy of Th. 2 stable, without variation in number of macrosetae. A row of microsetae occurs along entire posterior margin of Th. 2 (not displayed in figure); a_5, m_1, m_2, m_4, m_{4p}, and all posterior macrosetae (series Pi, Pa, Pm, and Pp) present (Fig. 10B). Chaetotaxy of Th. 3 variable: 8 macrosetae always present in zone M, 5 in zone L, and 3 in zone Pl (Fig. 3B); macroseta a_{5e}, m_4, a_{6i}, and p_{1i} usually present (Fig. 10C).

Legs. Trochanteral organ with triangular setal pattern and up to 30 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy variable. Abd. 1, with 4-8 macroseta (Fig. 10D), row of microsetae present along entire posterior margin (not displayed in figure). Abd. 2 with 4-6 macrosetae (Fig. 10E): a_2, m_3, m_{3e}, and m_5 always present; a_3 and m_{3ep} polymorphic. Abd. 3 chaetotaxy stable, with 7 macrosetae: a_1, a_2, a_3, m_3, am_6, pm_6, and p_6 (Fig. 10F). Abd. 4 inner macrosetae varying from 8-11 (Fig. 10G). Basal mucronal tooth slightly larger.

Remarks. *Entomobrya atrocincta* can be distinguished by the male or female color patterns as described above combined with the presence of macrosetae head S_{4i} and Abd. 3 a_1 and a_2 and the absence of macrosetae head ps_3 and Th. 2 m_5 (see Table 2 for additional diagnostic characters). The color pattern of female *E. atrocincta* is virtually indistinguishable from that of *E. multifasciata* and very similar to *E. intermedia*, and *E. nivalis*. Though these species may exhibit slight differences in color pattern, it is easier to differentiate them with the characters outlined in Table 3. Female *E. multifasciata* and *E. atrocincta* can be separated by the presence of head macroseta S_{4i} in *E. atrocincta* and its absence in *E. multifasciata*, and morphology of the labral papillae; *E. multifasciata* has 2-3 large seta or spine-like projections per papillae, whereas *E. atrocincta* has 3-4 small, seta or spine-like projections per papillae (Fig. 2).

The male form always has a unique orange color, but the distribution of purple pattern is variable. Christian and Bellinger (1998) report four discrete color forms. An additional male color form was collected during this study. The different color forms can commonly be found together within the same population. There are no significant morphological or COI DNA sequence differences between male forms and variation in color pattern (male and female) is attributed to intraspecific variation (Katz et al. 2015). The most common male form collected in this study (Fig. 9A) is somewhat similar to *E. clitellaria*. However, the two forms can be easily separated by chaetotaxy (Table 3).

Sexual dimorphism in this species has caused serious taxonomic confusion due to the similarity of female pattern to *E. multifasciata* and *E. nivalis*. Ramel et al. (2008) first described the sexual dimorphism of *E. atrocincta* from Greece and even noted that records of *E. multifasciata* may be misidentified *E. atrocincta* females. However, Jordana (2012) separated most European specimens and classified them as *E. nigrocincta* Denis based on chaetotaxy, synonymizing Ramel et al.'s (2008) descriptions with *E. nigrocincta*. Jordana (2012) attributes the sexual dimorphism (the same displayed by the specimens collected in North America and included in this study) to *E. nigrocincta* only, keeping the description by Christiansen (1958b) valid for *E. atrocincta*. Molecular data confirm that different color forms represent different sexes of the same species (Katz et al. 2015), demonstrating the presence of sexual dimorphism in North American *E. atrocincta*. However, species diagnosis remains unclear. Chaetotaxy outlined for both *E. atrocincta* and *E. nigrocincta* by Ramel et al. (2008) and Jordana (2012) do not match the specimens examined in this study (Table 4). The excessive intraspecific variation in chaetotaxy observed in these specimens raises concern about basing species diagnosis strictly on discrete chaetotaxic characters. Further molecular analysis of European populations is needed in order to elucidate the correct taxonomic status and distribution of these two species.

Table 3. Diagnostic characters to separate species within the nivalis complex: *E. atrocincta*, *E. multifasciata*, *E. intermedia*, and *E. nivalis*. Diagnostic characters of *E. clitellaria* are also included in relation to *E. atrocincta* due to similarity in color pattern.

Species	Head macroseta S_{4i}[1]	Th. 2 macroseta m_5[1]	Abd. 2 macroseta m_{3ep}[1]	Abd. 3 macroseta a_1[1]	Abd. 3 macroseta a_2[1]	Labral papillae[2]
E. atrocincta	1	0	0(1)[3]	1	1	0
E. multifasciata	0	0	0	1	1	0
E. intermedia	0	1	0	1	0	0
E. nivalis	1	1	1	1	0	0
E. clitellaria	1	1	1	0	1	1

[1] Character states: absent (0), present (1).

[2] Character states: multiple projections (0), single projection (1).

[3] Parentheses indicate a rarely observed state.

Table 4. Comparison between descriptions of *E. atrocincta* (and *E. nigrocincta*) provided by this study with those provided by Jordana (2012), Ramel et al. (2008), and Christiansen and Bellinger (1998). Characters represent the number of dorsal macrosetae present in fields (H1-A10) outlined by Jordana and Baquero (2005) and Jordana (2012). Clear differences between descriptions are highlighted in bold and underlined. Parentheses indicate a rarely observed state. Question marks indicate the characters were not included in the description.

Species description	Sexual dimorphism	H1	H2	H3	H4	H5	T1	T2	A1	A2	A3	A4	A5	A6–A10
E. atrocincta[1]	Yes	3	2(1)	0	3	2	2	3	2(1)	2(3)	1	2	1	8-11
E. nigrocincta[2]	Yes	3	1	0	**2**	2	2	3	1	2	1	**1**	1	9(8?)
E. atrocincta[3]	**No**	**2**	1	0	3	2(3)	2	3	2	3	1	2	1	**13**
E. atrocincta[4]	**No**	?	?	?	?	?	?	?	2	2	1	2	1	?

[1] Description of *E. atrocincta* specimens observed in the present study.

[2] Description of *E. nigrocincta* provided by Jordana (2012) and Ramel et al. (2008).

[3] Description of *E. atrocincta* provided by Jordana (2012).

[4] Description of *E. atrocincta* provided by Christiansen and Bellinger (1998).

The original description of *E. atrocincta* by Schött (1896) was based on the male form collected from California. Christiansen's (1958b) descriptions and methods for species delimitation placed heavy emphasis on the male genital plate, which may have led to his inadvertent omission of the female form. Given that the combination *E. atrocincta* Schött, 1896 has priority over *E. nigrocincta* Denis, 1923, and also because the holotype designated for *E. atrocincta* was collected in North America, the specimens along with the descriptions outlined in this study have been assigned to *E. atrocincta*.

Distribution. North America, Hawaii and possibly Europe. Records of *E. multifasciata* in North America and Hawaii (Christiansen and Bellinger 1992) are suspect due to their similarity to the female *E. atrocincta* color form. A considerable number of collections of *E. atrocincta* from Hawaii also include *E. multifasciata*, indicating the species is also sexually dimorphic in the Pacific Islands (Christiansen and Bellinger 1992).

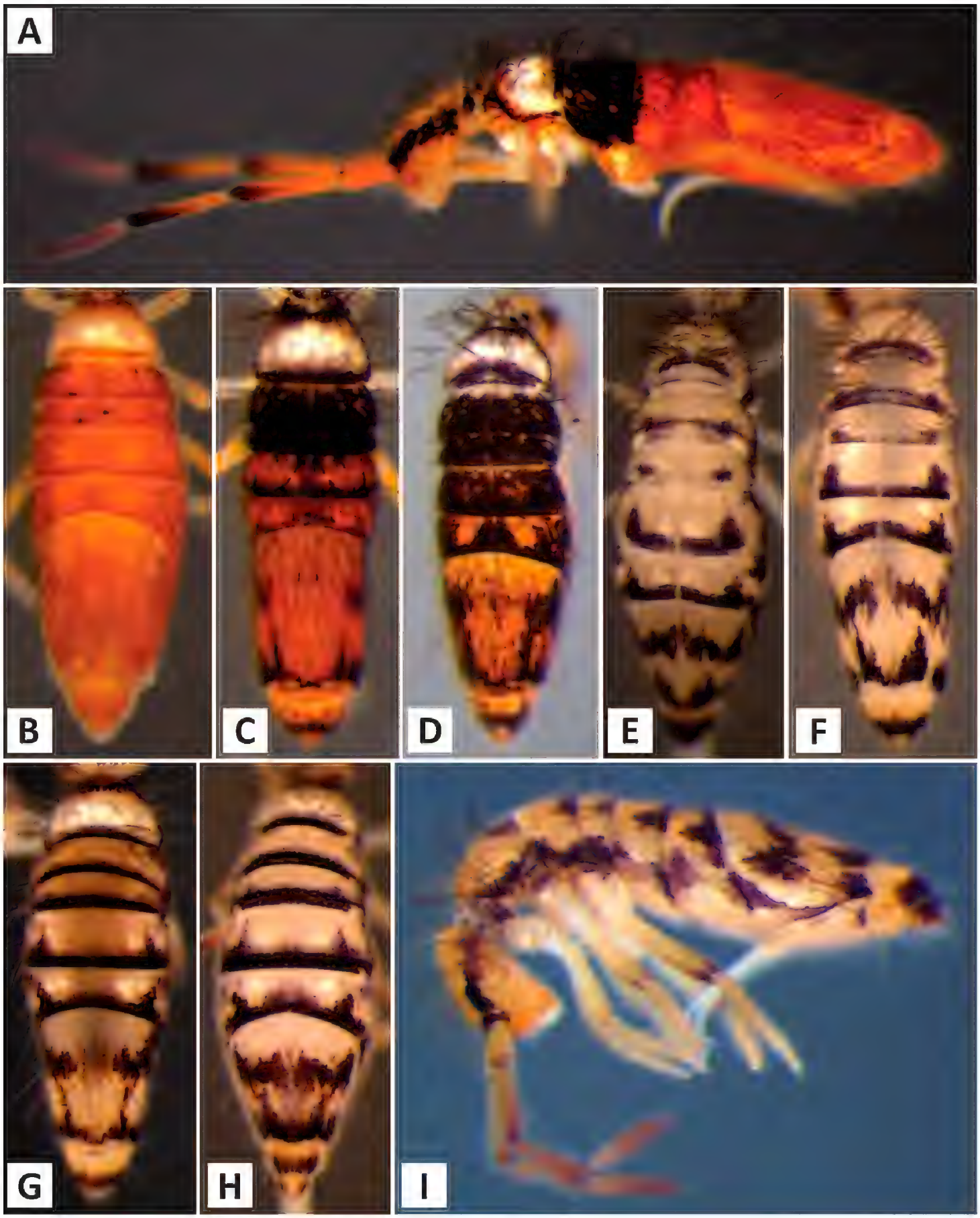


Figure 9. Color patterns of *Entomobrya atrocincta*: **A–D** Male color forms **E–I** Female color forms. All photographed specimens were collected from Champaign Co., IL.

Palearctic records of *E. atrocincta* may be *E. nigrocincta* provided by Jordana (2012). The distribution of *E. atrocincta* in North America is shown in Suppl. material 2: B.

Material examined. USA: 1♂ in vial, Alabama, Clay Co., Talladega National Forest, on CR6000-1 off of Hwy148 (33.19723,-86.06325), in moss on forest floor, 2.i.2012 (A. Katz & M. DuBray), AK12-5; 6♂ & 2♀ in vial, Illinois, Champaign

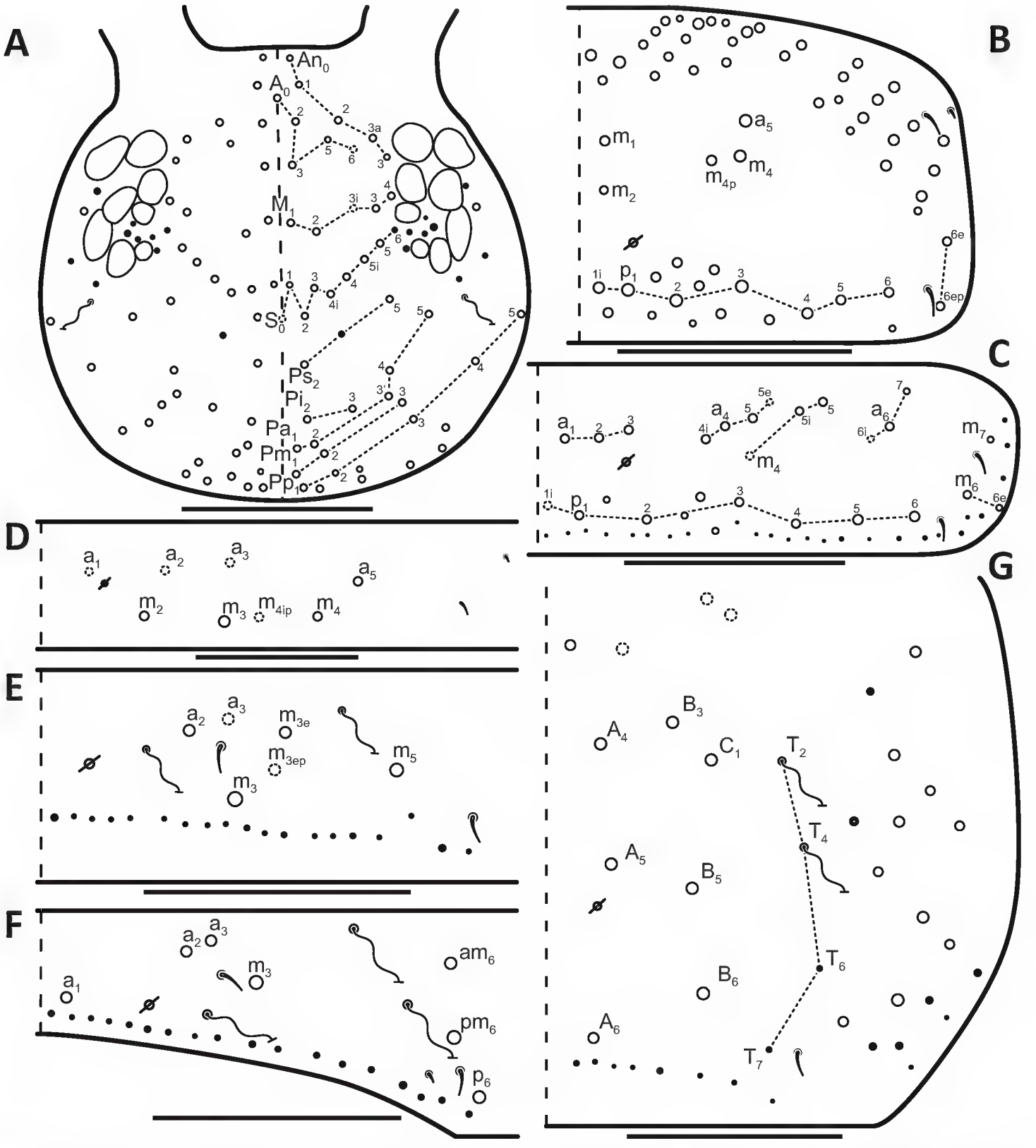


Figure 10. Dorsal chaetotaxy of *Entomobrya atrocincta*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), in bird nest, 8.v.2011, AK11-26; 2♂ on slides, 4♂ & 6♀ in vial, Illinois, Champaign Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), squirrel nest, 8.v.2011, AK11-27; 7♂ & 7♀ on slides, 50+♂ & 50+♀ in vial, Illinois, Champaign Co., Urbana, Natural Resource Building (40.10071,-88.22812), in leaf litter under bush by parking lot, 22.iv.2011, AK11-17; 1♂ on slides, Illinois, Champaign Co., Urbana, Meadowbrook Park (40.08063,-88.20828), in leaf litter, 14.iv.2012, AK12-24; 3♀ in vial, Illinois, Champaign Co., Urbana, South side of Natural Resource Building (40.10110,-88.22963),

in sweet gum seed pods and leaf litter, 23.iv.2011 (A. Katz & M. DuBray), AK11-15; 3♀ on slides, 20♂ & 10♀ in vial, Illinois, Champaign Co., Urbana, Natural Resource Building (40.10047,-88.22840), in leaf litter under bush along Pennsylvania Ave, 5.v.2011, AK11-25; 1♂ & 1♀ on slides, 50+♂ & 50+♀ in vial, Tennessee, Knox Co., University of Tennessee, Ag. Campus, Morgan Hall (36.01023,-83.93829), on moist fallen bark, April 2010 (E. C. Bernard); 1♂ & 1♀ in vial, Tennessee, Knox Co., Farragut, 12108 Ridgeland Drive (36.01023,-83.93829), in old bald-faced hornet nest, 2.iii.2008 (E. C. Bernard).

Entomobrya bicolor Guthrie, 1903

Figs 2, 11, 12, 13A, 39

Description. *Body shape and color pattern.* Body elongate and cylindrical. Only one reported color form in adults (Fig. 11A), dark brown or black pigment covering whole body except for a white band across Abd. 1, 2 and medial area of Abd. 3; Abd. 5 sometimes with 1+1 pale spots; legs, furcula, Abd. 6 pale, lacking all dark pigmentation; antennae lightly colored with brown or purple pigment, usually with a white area on distal half of 1st antennal segment. Juvenile pattern distinct from adult, yellow background without dark pigment except for eye patch; legs, furcula, Abd. 6 pale, lacking all dark pigmentation; antennae with light purple pigment. (Fig. 11B)

Head. Apical bulb of 4th antennal segment usually bilobed, rarely simple. Long differentiated smooth setae on ventral side of 1st antennal segment ≈ 3–4× short seta. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with a single seta or spine-like projection (Fig. 2). Labial papilla E with lateral appendage reaching just above tip of papilla. Labial triangle in one individual with 2 small supplementary ciliate microsetae internal to M1. Dorsal head chaetotaxy as in Figure 12A: macrosetae An'_0, A_6, S'_0, S_6, Ps_3, Ps_5, Pi_1, Pi_3, and Pm_{1i} always absent; An_{3a3} usually absent (present on one side in one specimen). Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax. Thoracic chaetotaxy abundantly developed, highly variable with many supplemental macrosetae. Th. 2 macrosetae p_{6e} and p_{6ep} absent and macrosetae m_{4i2}, m_{4i3} polymorphic (Fig. 12B). Th. 3 macrosetae a_{5e3}, m_4, m_{5p}, a_{6i}, a_7, and m_7 are absent (Fig. 12C). If present, m_7 is always a mesoseta. Chaetotaxy of zone Pm of both thoracic segments densely packed with many supplemental macrosetae, forming wing-like patches of posterior setae extending near anterior row, typical for species within the *E. bicolor* complex (Fig. 3D). Position of pseudopores on Th. 3 atypical for *Entomobrya*, displaced anteriorly and closer to macrosetae a_1, a_2, and a_3 than in other species.

Legs. Trochanteral organ with rectangular setal pattern and up to 37 setae. Unguis with 4 internal teeth; basal teeth enlarged and located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy highly developed. Row of microsetae along entire posterior margin present in all segments (not displayed in figure). Abd. 1 with 12

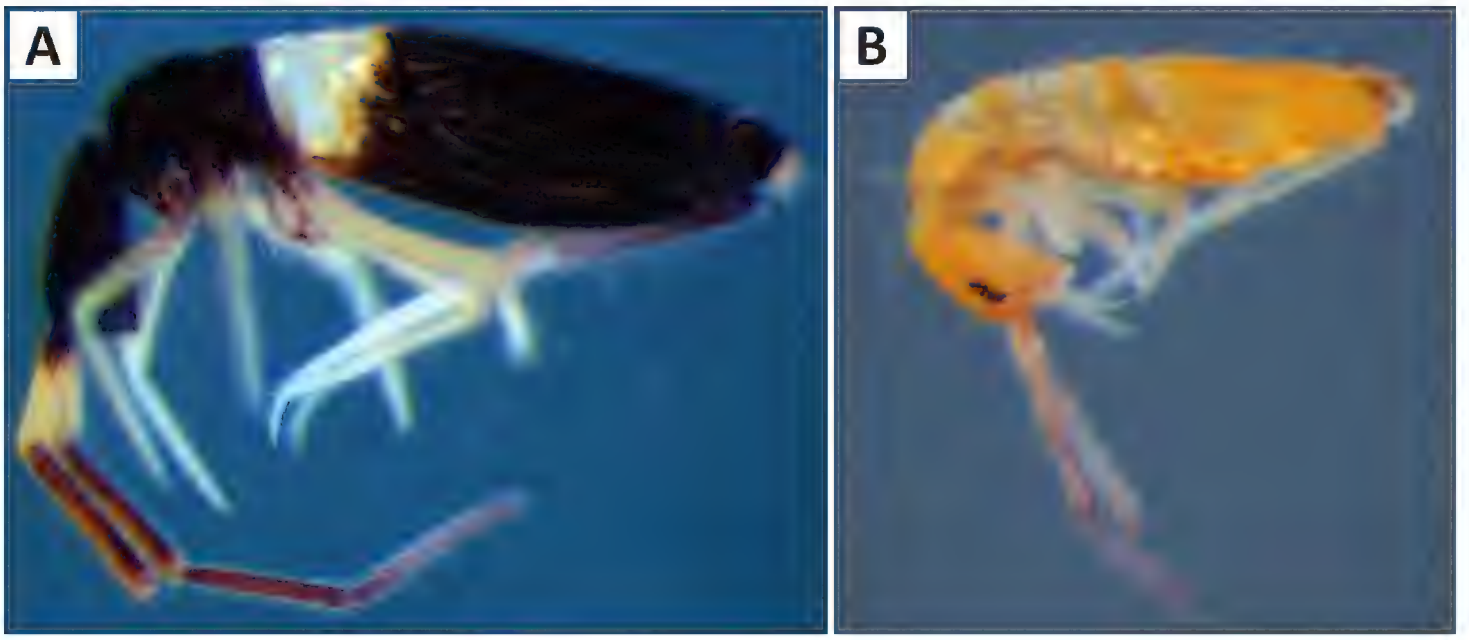


Figure 11. Color patterns of *Entomobrya bicolor*: **A** adult **B** young immature instar. Both photographed specimens were collected from Henderson Co., IL.

macrosetae (Fig. 12D). Abd. 2 with 10-11 macrosetae: a_2, a_3, m_3, m_{3e}, m_{3ep}, m_{3ep2}, m_{3ea}, m_5, p_5, and p_6 always present. Abd. 2 macroseta m_{3eai} sometimes present, m_{3ei} always absent (Fig. 12E). Abd. 3 macrosetae a_2, a_3, m_3, am_6, pm_6, p_5, p_6 present; a_1 always absent (Fig. 12F). Abd. 4 elongated, with at least 22 inner macrosetae (Fig. 12G), number of macrosetae extremely variable between individuals and within individuals (Fig. 13A). Position of pseudopores on Abd. 4 unstable, even varying with respect to macroseta and bothriotricha between sides on same individual (Fig. 13A). Mucronal teeth subequal; mucronal spine enlarged.

Remarks. *Entomobrya bicolor* adults can be easily distinguished by the unique color pattern described above combined with the absence of head macroseta Ps_5 (see Table 2 for additional diagnostic characters). Adults have a single, highly stable color form that is easily recognizable and perfectly acceptable for diagnosis (Fig. 11A). Molecular data from Katz et al. (2015) revealed that a small specimen found with *E. bicolor,* which was completely yellow and lacking all dark pigment, is the juvenile form of *E. bicolor* (Fig. 11B). The *E. bicolor* juvenile color form has never been reported in the literature. Unfortunately, it may be difficult to diagnose juvenile members of this species if collected without adults present in the sample.

Christiansen (1958b) placed this species in what he termed "the *E. bicolor* group"; a complex of three closely related species; *E. quadrilineata*, *E. decemfasciata*, and *E. bicolor*. This group is characterized by a highly elongate, cylindrical body, a relatively long furcula, antennae and legs, and by their abundant and highly variable chaetotaxy; many duplicate, supplementary macrosetae, distinctive and augmented setal patterns in thoracic zone Pm, expanded chaetotaxy of Abd. 4, and high levels of asymmetry (Fig. 13A). The extreme setal variation obscures potentially informative characters and results in a lack of discrete, useful diagnostic chaetotaxy between species in this complex. The absence of head macroseta Ps_5 is the only character (other than color pattern)

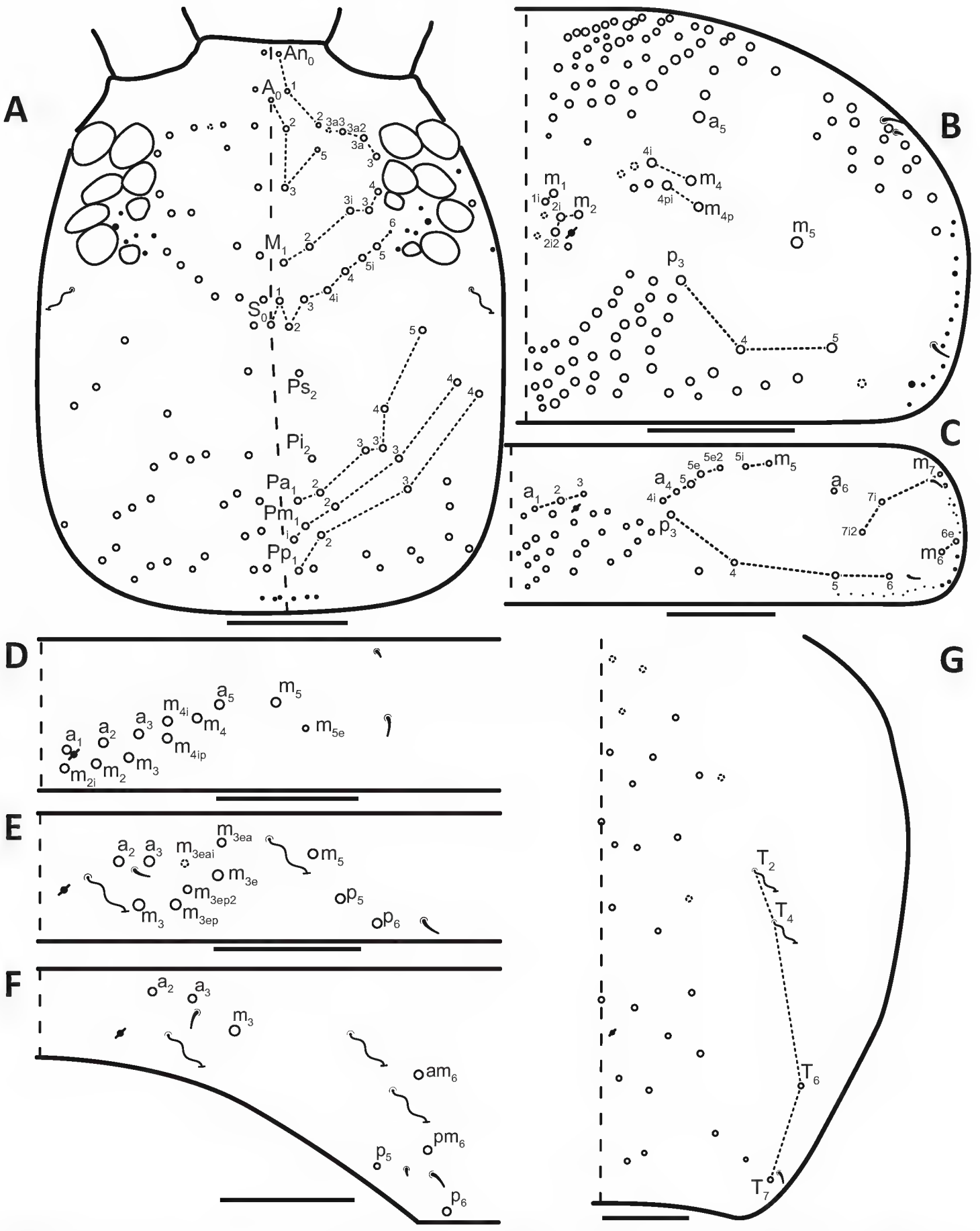


Figure 12. Dorsal chaetotaxy of *Entomobrya bicolor*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

that differentiates *E. bicolor* from *E. decemfasciata* and *E. quadrilineata*. Color pattern is critical for species diagnosis within this complex. See Table 5 for a summary of the important diagnostic characters to separate species in this complex.

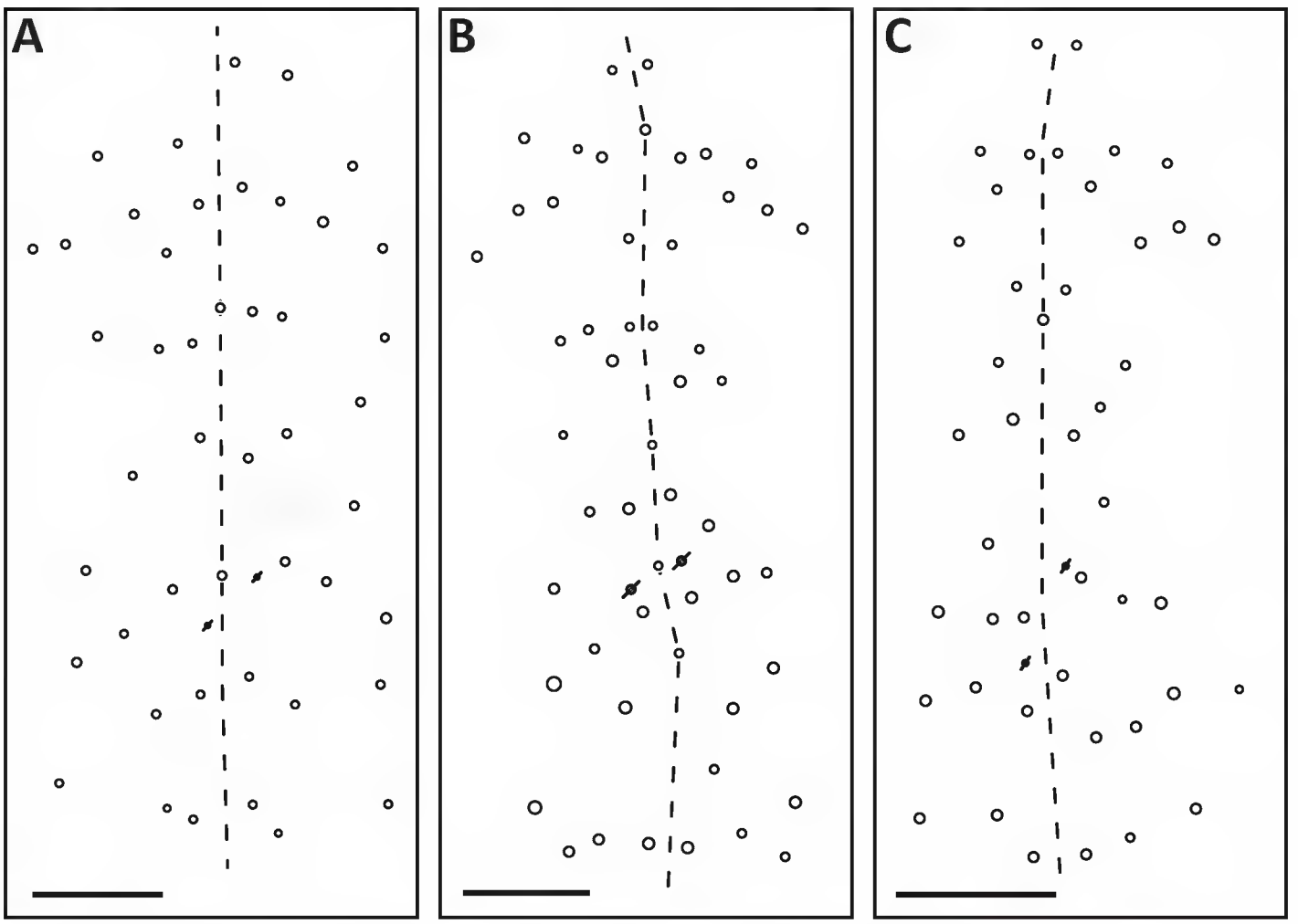


Figure 13. Macrosetae variation and asymmetrical polymorphism of Abd. 4 in the *E. bicolor* complex: **A** *E. bicolor* **B** *E. decemfasciata* **C** *E. quadrilineata*. Only medial macrosetae (those which occur between bothriotricha T_2 and T_4) are included in diagram. The dotted line represents the medial division of Abd. 4. Scale bars = 100 μm.

Table 5. Important diagnostic characters for the separation of species within the bicolor complex: *E. bicolor*, *E. decemfasciata*, and *E. quadrilineata*. Character states: absent (0), present (1).

Species	Head macrosetae Ps_5	Parallel, longitudinal bands on thorax
E. bicolor	0	0
E. decemfasciata	1	0
E. quadrilineata	1	1

Distribution. Endemic to North America (Suppl. material 2: C).

Material examined. USA: 9 on slides, 6 in vial, Illinois, Henderson Co., Big River State Forest (41.03435,-90.91620), vacuum sand prairie, 8.vi.2011 (C. H. Dietrich).

Entomobrya citrensis Katz & Soto-Adames, sp. n.

http://zoobank.org/4C33A32D-EFF4-4B53-8880-2C9C3844076C

Figs 2, 14, 15, 16A

Etymology. This species is named after the locality it was collected in: Citrus County, Florida. Citrensis is Latin for "from the place of citrus".

Figure 14. Color pattern of *Entomobrya citrensis* sp. n. Lateral (**A**) and dorsal (**B**) views of specimen collected from Citrus Co., FL.

Type material. *Holotype*, ♂, USA: Florida, Citrus County, Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), beating vegetation, 12.viii.2011 (A. Katz & J. Cech), AK11-134.

Paratypes, USA: 1 on slide, Florida, Citrus Co., Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), under bark, 12.viii.2011 (A. Katz & J. Cech), AK11-136; 1 on slide, Florida, Citrus Co., Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), beating vegetation and in leaf litter, 5.i.2014 (A. Katz & M. DuBray), AK14-1.

Description. *Body shape and color pattern.* Body dorso-ventrally flattened. Length up to 1.4 mm. Color pattern monomorphic (Fig. 14): background white, or slightly yellow, with black and traces of dark brown pigmentation forming transversal bands and spots. Thorax dorsal pigmentation patchy and irregular. Abd. 2 usually with 2 dark lateral spots. Posterior margin Abd. 2 and the anterior margin of Abd. 4 with a dark transverse band. Abd. 4 with a conspicuous irregular dark transverse band medially. The antennae are completely pigmented purple.

Head. Apical bulb of 4th antennal simple. Long differentiated smooth setae on ventral side of 1st antennal segment ≈2.5× short setae. Prelabral setae with very fine ciliations that look smooth at low magnification under light microscopy. Ornamentation of the distal margin of the labral papillae with 3-4 spine-like projections on the inner papillae and 2 spine-like projections on the external papillae. Lateral appendage of labial papilla E slightly curved, relatively thin, reaching just below tip of papilla. Dorsal head chaetotaxy slightly reduced (Fig. 15A): macrosetae An'_0, A_6, M_3, M_{3i}, S'_0, S_1, Pi_1, Pa_2, Pa_3, Pm_2, and Pm_{1i} always absent; An_{3a3} present, asymmetrically absent in one specimen. Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax. Thoracic chaetotaxy reduced and fixed, no macrosetae variation observed. Row of microsetae along entire posterior margin of Th. 2 and Th. 3 (not displayed in figures). Th. 2, with a_5 and posterior macrosetae p_4, p_5, p_6, p_{6e}, and p_{6ep} (Fig. 15B). Th.

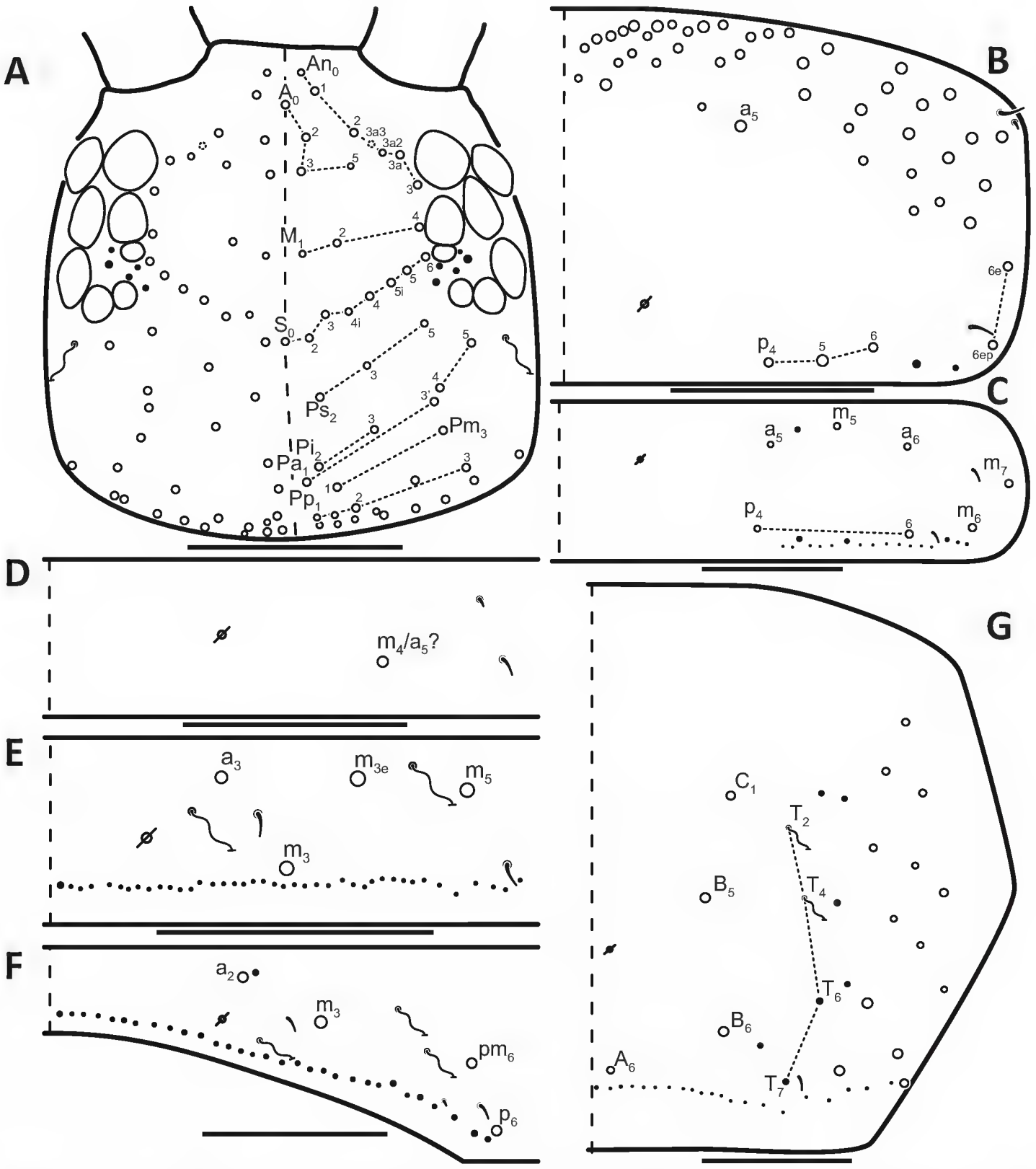


Figure 15. Dorsal chaetotaxy of *Entomobrya citrensis* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

3, with 7 macrosetae present: a_5, a_6, m_5, m_6, m_7, p_4, and p_6; macrosetae a_1, a_2, and a_3 absent (Fig. 15C). Zone Pm (Fig. 3) without macrosetae.

Legs. Trochanteral organ with triangular setal pattern and up to 14 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length (Fig. 16A).

Abdomen. Abdominal chaetotaxy reduced and stable; no macrosetae variation observed. Abd. 1 with 1 macroseta only (Fig. 15D). Abd. 1 row of microsetae along

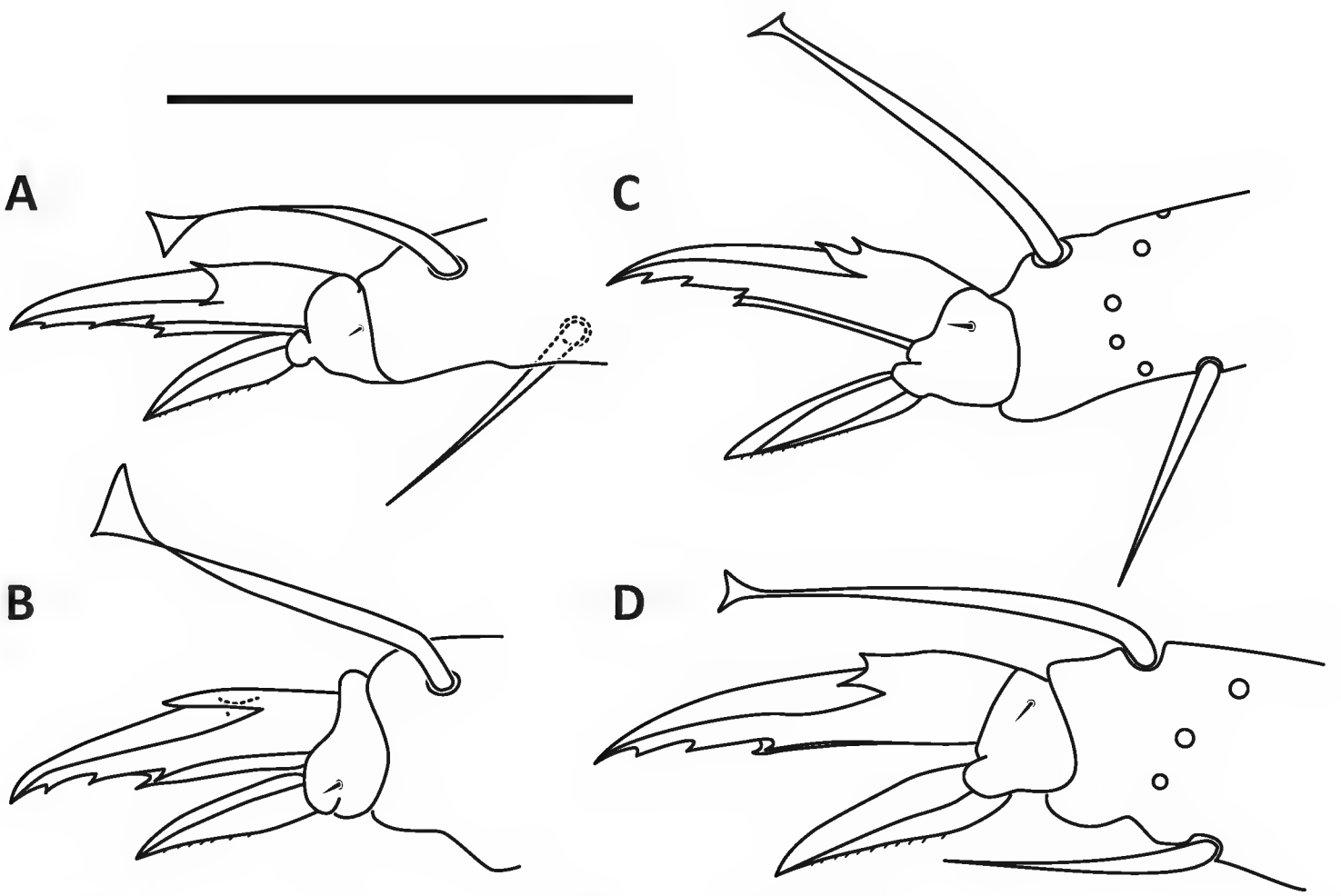


Figure 16. Unguis (top claw) and unguiculus (bottom claw) on 3rd pair of legs. **A** *Entomobrya citrensis* sp. n. **B** *Entomobrya jubata* sp. n. **C** *Entomobrya neotenica* sp. n. **D** *Entomobrya unifasciata* sp. n.

posterior margin is present (not displayed in figure). Abd. 2 with 4 macrosetae: a_3, m_3, m_{3e}, and m_5 (Fig. 15E). Abd. 3 with 4 macrosetae: a_2, m_3, pm_6, and p_6; mesosetae a_{2a} sometimes present, inserted slightly anterior and exterior to a_2; socket of a_{2a} large, resembling that of macroseta a_3, but its close proximity to a_2 suggests a duplication of a_2 rather than homologous to a_3 (Fig. 15F). Abd. 4 with 4 inner macrosetae (Fig. 15G). Mucronal teeth subequal.

Remarks. *Entomobrya citrensis* sp. n. can be distinguished by its color pattern in combination with the absence of Th. 2 macrosetae m_2 and m_5, Abd. 2 a_2 and m_{3ep}, and the presence of Abd. 2 a_3 (see Table 2 for additional diagnostic characters).

This species is closely related to *E. assuta*; both have highly compressed, or dorsoventrally flattened bodies, reduced chaetotaxy, and similar color patterns. Head macroseta Ps_3 and Abd. 2 macroseta a_3 are both present in *E. citrensis* sp. n., but are absent in *E. assuta*. These species can also be separated by color pattern and morphology of the labral papillae (Fig. 2).

See remarks for *E. assuta* for additional diagnosis information. Only a few specimens of *E. citrensis* sp. n. were observed from one locality. Additional sampling may reveal more variation in color pattern or chaetotaxy.

Distribution. Endemic to North America. Reported from a single locality: Chassahowitzka National Wildlife Refuge in Citrus County, Florida (Suppl. material 2: D).

Entomobrya clitellaria Guthrie, 1903

Figs 2, 17, 18, 39

Description. *Body shape and color pattern*. Body dorso-ventrally flattened. Dimorphic color pattern, unrelated to sex (Fig. 17): dark blue, purple, or black pigment covers Th. 3 and Abd. 1-3. Abd. 4 usually white, yellow, or orange with variable levels of pigment ranging from an irregular medial transverse band, randomly distributed irregular patches, or completely pigmented. Th. 2 always white, with a dark band along the anterior margin; medial area of Th. 2 almost transparent in some specimens and internal fat bodies can be observed through the cuticle under a dissecting microscope. Darker specimens with head mostly covered by dark pigment. Antennae usually entirely covered by purple pigment, but some specimens have a mixture of orange, brown, and purple coloration.

Head. Apical bulb of 4th antennal segment usually simple, sometimes bilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with a single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E slightly curved, relatively thin, nearly reaching tip of papilla. Labial triangle in one individual with 1 small supplementary ciliate microsetae internal to M1. Dorsal head chaetotaxy (Fig. 18A) with macrosetae An'_0, A_{3a3}, M_{3i}, S'_0, S_6, and Ps_3 always absent; An_{3a2} usually present; Pi_1, Pm_1, Pm_{1i}, and Pp_2 present or absent. Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax. Thoracic chaetotaxywell-developed, with some slight variation. Th. 2 macrosetae m_{4i3} absent, m_{1i} usually present (Fig. 18B). Th. 3 macrosetae a_{5e3}, m_4, m_{5p}, and a_7 absent (Fig. 18C). Both thoracic segments with many supplemental macrosetae in zone Pm (18B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 26 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy highly developed. Abd. 1 with 8-13 macrosetae (Fig. 18D). Abd. 2 macroseta a_2, a_3, m_3, m_{3ep}, m_{3e}, and m_5 present; M_{3ei} and M_{3ea} usually present and M_{3eai} usually absent (Fig. 18E). Abd. 3 macrosetae a_2, a_3, m_3, am_6, pm_6, and p_6 present; a_1 always absent (Fig. 18F). Abd. 4 chaetotaxy stable, with 7 macrosetae between bothriotrichal complexes (Fig. 18G). Mucronal teeth subequal.

Remarks. *Entomobrya clitellaria* can be distinguished by the combination of color pattern, absence of macroseta head S'_0 and Abd. 3 a_1 and presence of head Ps_5, Th. 2 m_5, Abd. 2 m_{3ep}, and Abd. 3 a_2 (see Table 2 for additional diagnostic characters). This species has a relatively conspicuous and diagnostic color pattern, but may be confused with the male *E. atrocincta*. However, there are obvious differences in their morphology outlined in Table 3. Christiansen and Bellinger (1998) described six different color forms, but after extensive examination of material collected for this study, it was determined that many (but not all) of the color forms they described were most likely variants within a continuous gradient of color pattern variation, without clear genetic isolation (Katz et al. 2015). The two lighter

Figure 17. Color patterns of *Entomobrya clitellaria*. Photographed specimens are from the following localities: **A** Monroe Co., IL **B** Jasper Co., IL **C** Kankakee Co., IL **D** Monongalia Co., WV.

forms (labeled E and F in Christiansen and Bellinger 1998) proved to be elusive and were not collected during this study.

This species is closely related to *E. jubata* sp. n., but can be easily separated by color pattern, chaetotaxy, and morphology of the labral papillae. The absence of head macrosetae S'_0 and the presence of head macrosetae Ps_5 separate *E. clitellaria* from *E. jubata* sp. n. Labral papillae morphology also differs between these species: *E. jubata* sp. n. has two or three seta or spine-like projection on each papilla, while *E. clitellaria* only has one seta or spine-like projection per papilla (Fig. 2).

Distribution. Endemic to North America (Suppl. material 2: E).

Material examined. USA: 1 on slide, Florida, Taylor Co., Econfina State Park (30.0656,-83.91066), under bark, 9.viii.2011, AK11-116; 1 in vial, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14462,-88.16543), on bushes and low-lying shrubs, 7.vii.2011, AK11-60; 1 on slide, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14391,-88.16468), under bark, 10.ix.2009 (F. Soto-Adames); 1 in vial, Illinois, Coles Co., Fox Ridge State Park (39.40248,-88.14893), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-81; 3 on slides, 20 in vials, Illinois, Jasper Co.,

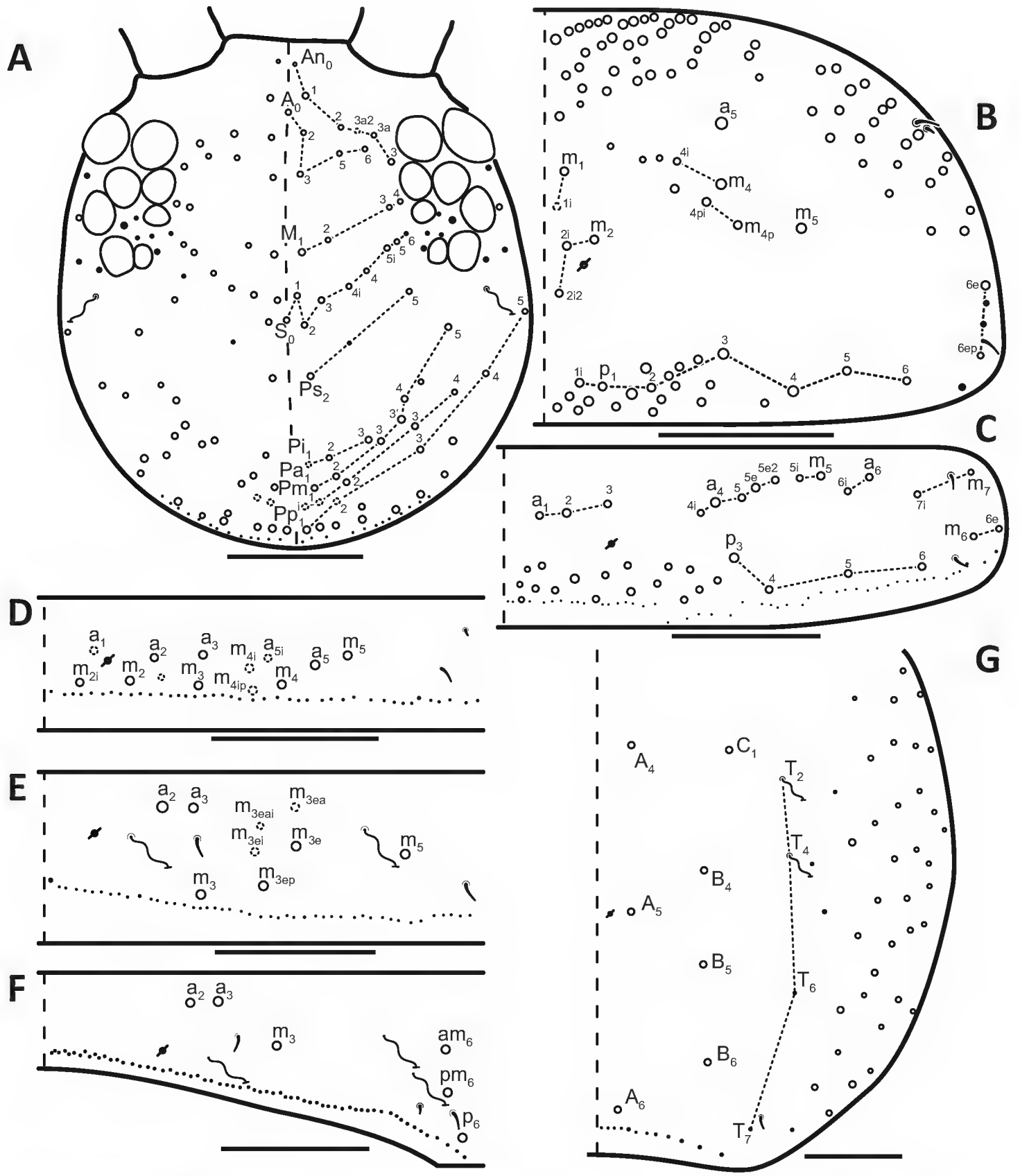


Figure 18. Dorsal chaetotaxy of *Entomobrya clitellaria*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

Sam Parr State Fish and Wildlife Area (39.03275,-88.12474), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-73, AK11-74 & AK11-75; 1 in vial, Illinois, Jasper Co., Sam Parr State Fish and Wildlife Area (39.03275,-88.12474), in leaf litter, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-77; 1 on slide, 1 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), under bark, 26.viii.2011, AK11-142; 1 in vial, Illinois, Jo Davies Co., S Blanding Rd, Stevenson Property (42.29895,-

90.36967), under bark, 27.viii.2011, AK11-148; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from bird nest, 10.iv.2011, AK11-6; 4 on slides, Illinois, Monroe Co., Bat Sump Cave, Berlese of bark, 1-3.xi.2009 (S. Taylor & F. Soto-Adames), sjt09-130; 4 on slides, 50+ in vial, Illinois, Piatt Co., Lodge County Forest Preserve Park (40.06709,-88.56596), dead logs, cavity in tree, and under bark, 23.vii.2011, AK11-100; 2 on slides, 10 in vial, Illinois, Pope Co., Bell Smith Springs (37.51927,-88.65738), leaf litter, 24.ix.2011, AK11-166; 2 on slides, Illinois, Vermilion Co., Kickapoo State Park (40.16576,-87.74746), on rotten log, 30.xi.1989 (F. Soto-Adames); 1 in vial, Illinois, Vermilion Co., Kennekuk Cover County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), aspirated from bushes, 16.vi.2011 (A. Katz & F. Soto-Adames), AK11-59; 1 in vial, Michigan, Ingham Co., Michigan State University, Baker Wdlt. (42.66527,-84.36264) under bark of standing dead pine, 24.vii.2008 (E. C. Bernard), BW-11; 1 on slide, 3in vial, Michigan, St. Clair Co., Algonac State Park (42.65447,-82.52430), under bark of recently fallen maple, 25.vii.2008 (E. C. Bernard), ASP-15; 1 on slide, West Virginia, Monongalia Co., Tyrone (39.62746,-79.86567), under collected on the lower branches of eastern hemlock, 19.vii.2005 (R. M. Turcotte), ID#44-GE-21-5-4-A.

***Entomobrya decemfasciata* (Packard), 1873**

Figs 2, 3D, 13B, 19, 20, 39

Description. *Body shape and color pattern.* Body very elongate and cylindrical with mesothorax forming a slight hump behind head. Color pattern remarkably variable with continuous variation and many intermediate forms (Fig. 19). Typical pattern without thoracic bands but with 2-4 irregular, angled bands on lateral margins of abdomen. Color also variable ranging from white, yellow, orange, or sometimes light blue or purple background with black, dark blue, or brown pigment forming bands. Dark bands outlining posterior and lateral margins of Th. 2 and Th. 3 sometimes present. Apex of femora usually with a dark patch. Head either entirely blue or purple, or lacking all pigment except for the eye patches. Dark patches of pigment usually occur on distal end of antennal segments 2-4. Juveniles usually with light blue pigment background and faint brown abdominal banding.

Head. Apical bulb of 4th antennal segment usually bilobed or simple, but with up to 6 distinct lobes. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E slightly curved, almost twice as long as papilla. Labial triangle chaetotaxy slightly irregular and atypical for this genus: M1, r, E, L1, L2 ciliate; r significantly smaller than other setae; A1-A5 smooth; sometimes 2 additional ciliate setae inserted internal to M1 and A1, respectively, often relatively difficult to observe. Post labial setae abundant, all type 5. Dorsal head macrosetae (Fig. 20A) An'_{0}, An_{3a3}, S'_{0}, S_{6}, Ps_{3}, and Pm_{1i} absent; A_{6} sometimes present. Eyes G and H small and subequal. Eye patch with 5 setae.

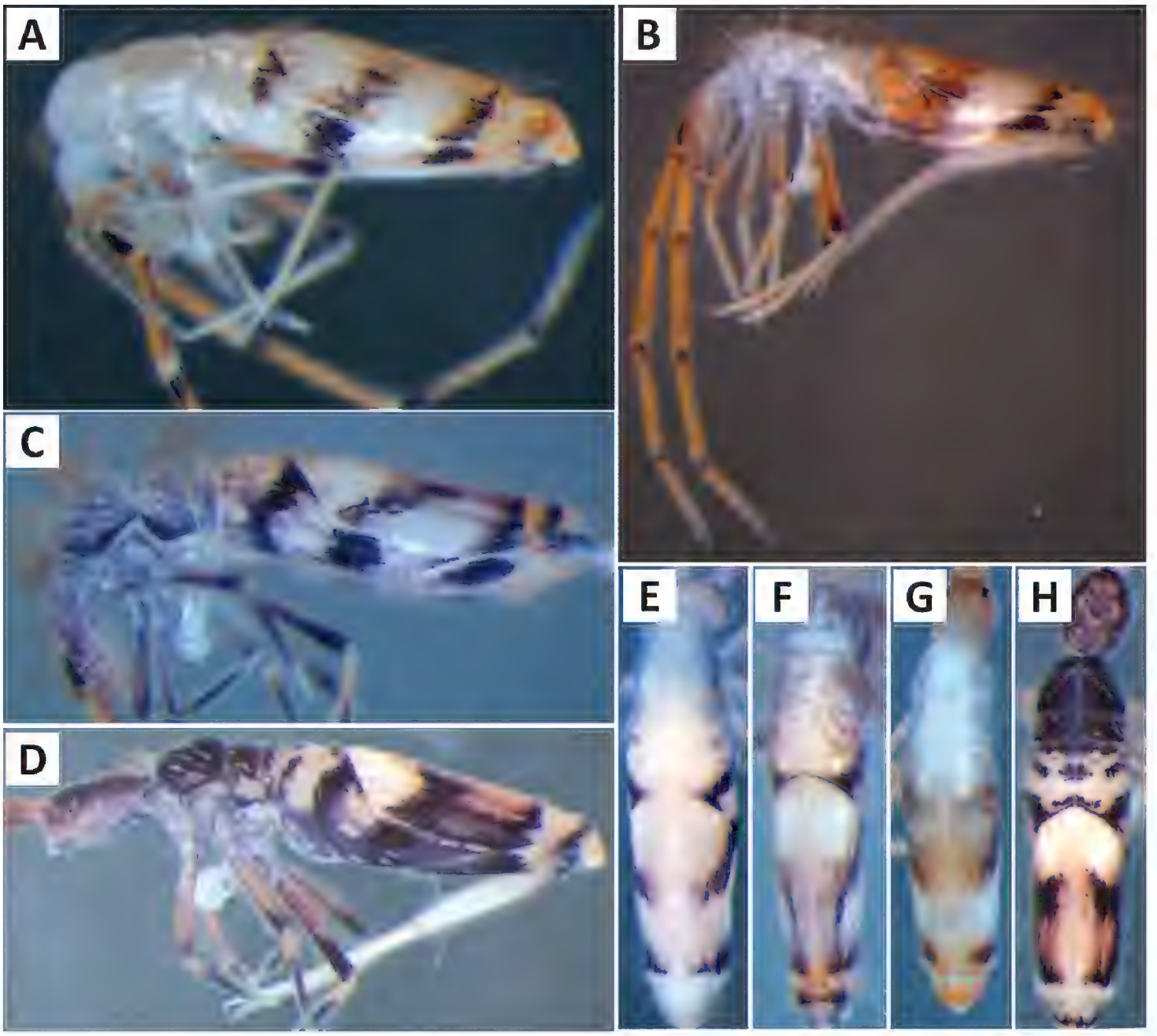


Figure 19. Color patterns of *Entomobrya decemfasciata*. Photographed specimens are from the following localities: **A** Stewart Co., TN **B** Stewart Co., TN **C** Clay Co., AL **D** Sevier Co., TN **E** Sequatchie Co., TN **F** Clay Co., AL **G** Union Co., IL **H** Sevier Co., TN.

Thorax. Thoracic chaetotaxy developed, highly variable, with many supplemental macrosetae. Th. 2 macrosetae p_6, p_{6e} and p_{6ep} absent. Macrosetae in Zone A so abundant that usually merge with medial macrosetae, forming a single, large patch of setae (Figs 3D; 20B). Th. 3 macrosetae a_{5e3}, m_4, m_{5p}, a_{6i}, a_7, and m_7 absent (Fig. 20C). Macrosetae in Zone Pm also very abundant on both thoracic segments, with many supplemental macrosetae present forming wing-like patches of posterior setae extending near the anterior row (Fig. 3D). Insertion of pseudopore on Th. 3 different from most *Entomobrya*, displaced anteriorly, very close to macrosetae a_1, a_2, and a_3. Additional duplicate or supplementary setae often form columns internal to a_1.

Legs. Trochanteral organ with rectangular setal pattern and up to 86 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy extensively developed. Row of microsetae along entire posterior margin present in all segments (not displayed in figure). Abd. 1 with

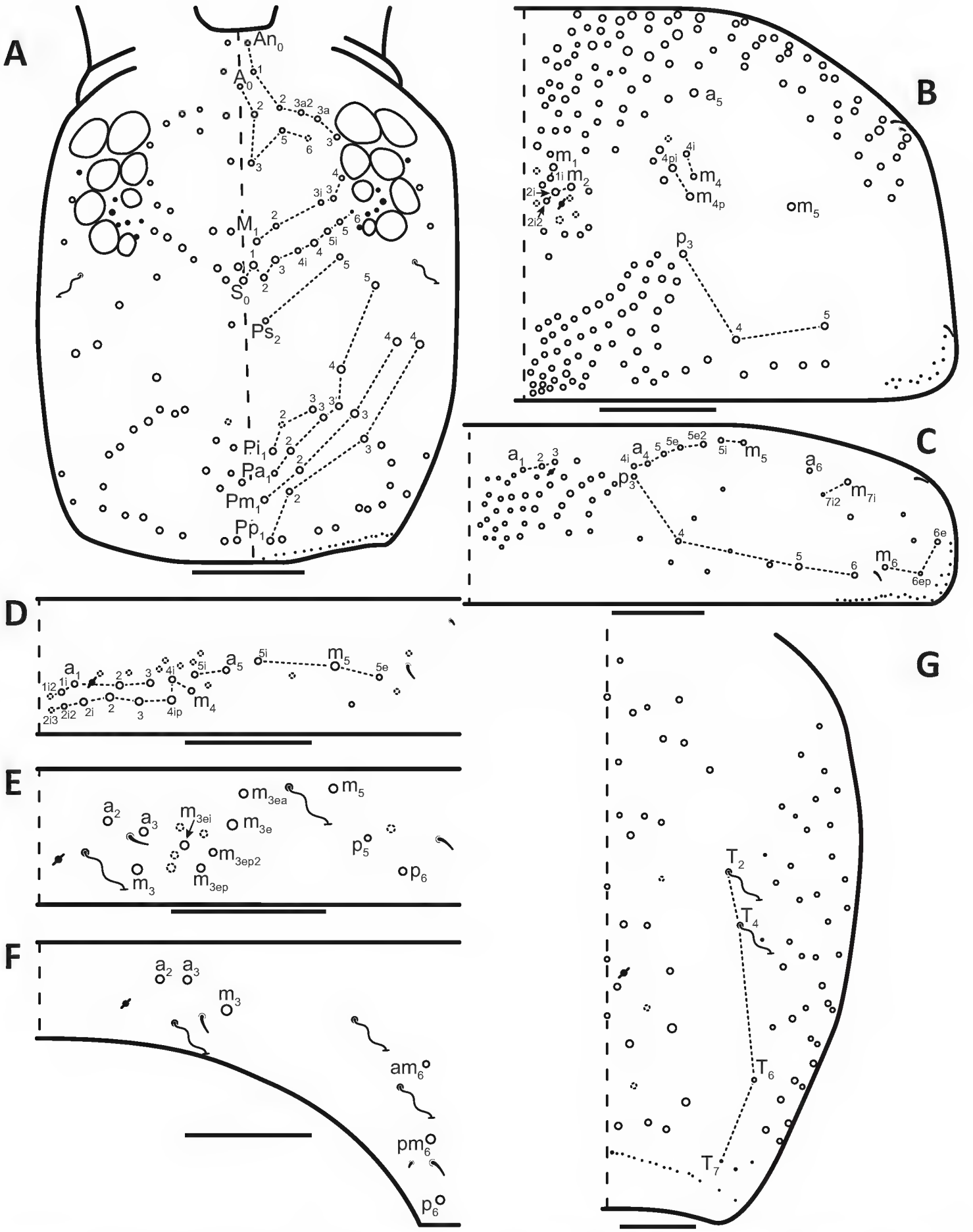


Figure 20. Dorsal chaetotaxy of *Entomobrya decemfasciata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

16-30 macrosetae (Fig. 20D). Abd. 2 macroseta M_{3ei} always present, with up to 4 additional supplementary macrosetae (possibly including M_{3eai}) internal to m_{3e}; macrosetae a_2, a_3, m_3, m_{3e}, m_{3ep}, m_{3ep2}, m_{3ea}, m_5, p_5, and p_6 present (Fig. 20E). Abd. 3 with

macrosetae a_2, a_3, m_3, am_6, pm_6, and p_6; a_1 absent (Fig. 20F). Abd. 4 very elongated, with at least 25 inner macrosetae (Fig. 20G), but number of macrosetae extremely variable between individuals and even within a single individual (Fig. 13B). Insertion of pseudopores on Abd. 4 varying with respect to macroseta and bothriotricha even in same individual (Fig. 13B). Basal mucronal tooth enlarged.

Remarks. *Entomobrya decemfasciata* can be distinguished by the absence of parallel, longitudinal bands on the thorax, the presence of 2-4 irregular, angled bands on lateral margins of abdomen, and the presence of head macroseta Ps_5 (see Table 2 for additional diagnostic characters). This species belongs to the *E. bicolor* group (see remarks for *E. bicolor*), and exhibits high levels of variation in both color pattern and chaetotaxy. *E. decemfasciata* is perhaps the most setaceous species of *Entomobrya* reported for North America, clothed in hundreds of macrosetae. This abundant and hyper-variable chaetotaxy provides few characters to differentiate *E. decemfasciata* from *E. bicolor* and *E. quadrilineata*. However, clear differences in color pattern can be observed between these species; *E. bicolor* lacks band or stripes, *E. quadrilineata* always has two parallel longitudinal stripes extending from the thorax through Abd. 2 and *E. decemfasciata* never has bands or stripes on the thorax. Table 5 details important diagnostic characters to separate species within this species complex.

The considerable variation in chaetotaxy and color pattern and relatively high molecular divergences between *E. decemfasciata* color forms suggest the presence of a cryptic species complex (Katz et al. 2015). However, the lack of diagnostic morphological characters between color forms does not allow the circumscription of new species at this time. More specimens and additional molecular and morphological analyses are needed for further action.

This species has a long history of taxonomic issues (Christiansen 1958b) and its separation from *E. quadrilineata* only became evident after a thorough molecular analysis (Katz et al. 2015). Christiansen (1958b) separated *E. decemfasciata* from *E. quadrilineata* using color pattern, male genital plate, antennal ratios, and later added chaetotaxy in *The Collembola of North America* (Christiansen and Bellinger 1998). High variability in color pattern and chaetotaxy, deformation of antennae after slide mounting, and the difficulty of discerning the morphology of setae on the genital plate, lessens the utility of these characters for diagnosis. Christiansen and Bellinger (1998) even suggested *E. quadrilineata* may be a variant form of *E. decemfasciata* and seemed uncertain regarding differences in chaetotaxy. Furthermore, Christiansen (1958b) reported three distinct color forms for *E. decemfasciata* (all of which had a V-shape or two angled lateral bands on Abd. 2 and lacking thoracic stripes) and three distinct color forms for *E. quadrilineata*, one of which also lacks thoracic stripes. During the course of this study, many color forms were collected, including forms both with and without thoracic stripes occurring together in the same sample. These sympatric forms lacked a V-shape or two lateral angled bands on the 2rd abdominal segment, and, following Christiansen and Bellinger's (1998) concept, were diagnosed as *E. quadrilineata*. However, large molecular distances made it apparent that forms with and without thoracic stripes were separate species (Katz et al. 2015), warranting further diagnostic inquiry. Type specimens from the Illinois Natural His-

tory Survey were attained for both *E. quadrilineata* and *E. decemfasciata*. Though both types, preserved in alcohol, were in relatively poor condition, two longitudinal thoracic stripes were clearly observed on the *E. quadrilineata* specimen (Suppl. material 3: A ,B). Thoracic banding was not observed on the type specimen of *E. decemfasciata* (Suppl. material 3: C). Based on molecular evidence (Katz et al. 2015) and the observations of types, specimens with the morphology corresponding to both *E. quadrilineata* and *E. decemfasciata* can be diagnosed by the presence or absence of parallel thoracic longitudinal stripes. Christiansen's (1958b) report of an *E. quadrilineata* color form lacking thoracic stripes may have been a case of misidentification of *E. decemfasciata*. If individuals with and without longitudinal bands were collected together, similar morphology and abdominal pigmentation may have led him to conclude that they were both *E. quadrilineata*. However, we now know that both species can occur in sympatry.

Distribution. Endemic to North America (Suppl. material 2: F).

Material examined. USA: *"Type material"*, 1 on slide, Knoxville, Tennessee (Dr. Curtis), INHS Cat. No. 528,321; *Cotype*, 1 on slide, no locality information; 1 on slide, 17 in vial, Alabama, Clay Co., Talladega National Forest, CR-7 & Hwy148 (33.19723,-86.06325), leaf litter, 2.i.2012, AK12-2; 3 on slides, 20 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, vestibule of back high entrance, moist leaf litter along the wall and some dry litter from the center of the vestibule, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 16 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, humid and cool litter in side niche near main entrance, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 4 on slides, 2 in vial, Tennessee, Sequatchie Co., Moonshadow (35.64167,-83.76359), forest leaf litter, 14.i.1997 (M. M. Gibbs); 2 on slides, Tennessee, Sevier Co., Great Smoky Mountains National Park, ATBI plot, Goshen Prong (35.67961,-83.50021), malaise 22 83 32 34 35 36 38, 12.xi.2001-5.xii.2001 (Parker, Stocks & Peterson); 2 on slides, 3 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-105; 2 on slides, 50+ in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd. (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-107; 2 on slides, 11 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area (36.53830,-87.91428), leaf litter, 7.viii.2011, AK11-108.

Entomobrya intermedia Brook, 1883

Figs 2, 21, 22, 39

Description. *Body shape and color pattern*. Body oval and cylindrical. Color pattern monomorphic (Fig. 21): yellow background with black, dark brown or purple pigment forming two incomplete, broken, longitudinal bands from Th. 2 through Abd. 3 and a conspicuous W-shaped mark on Abd. 4. Usually with dark pigment covering the lateral margins of the head. Antennae light brown or purple becoming increasingly dark towards the apex.

Figure 21. Color pattern of *Entomobrya intermedia*. Lateral (**A**) and dorsal (**B**) views of specimen collected from Chester Co., PA.

Head. Apical bulb of 4th antennal segment simple. Long differentiated smooth setae on ventral side of 1st antennal segment ≈5× short setae. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2-4 small spine-like projections (Fig. 2). Lateral appendage of labial papilla E slightly curved, thin, nearly reaching tip of papilla. Dorsal head chaetotaxy (Fig. 22A) with macrosetae An'_0, A_{3a2}, A_{3a3}, M_{3i}, S'_0, S_{4i}, S_6, Ps_3, Pi_1, and Pm_{1i} absent; an additional macroseta external to A_3 present in some specimens; S_1 and Pm_1 usually present, but may be asymmetrical. Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax: Thoracic chaetotaxy well-developed and relatively stable. Th. 2 macrosetae a5, m_1, m_2, m_{2i}, m_4, m_{4p}, m_{4i}, and m_5 present (Fig. 22B). Lateral sensilla obscured in all specimens examined. Posterior macrosetae (series P) present. Th. 3 macrosetae a_1, a_2, a_3, a_4, a_{4i}, a_5, a_6, m_4, m_5, m_{5i}, and m_6 present (Fig. 22C). Posterior macrosetae (series P) present. The chaetotaxy of zone Pm with a moderate number of supplemental macrosetae in both thoracic segments (Fig. 22B,C). Typical sensilla present (not shown in figure).

Legs. Trochanteral organ with triangular setal pattern and up to 31 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy stable. Abd. 1 with 7 macrosetae (Fig. 22D). Abd. 2 macroseta a_2, a_3, m_3, m_{3e}, and m_5 present (Fig. 22E). Abd. 3 with macrosetae a_1, m_3, am_6, pm_6, and p_6 (Fig. 22F). Abd. 4 with 9-10 inner macrosetae (Fig. 22G). Mucronal teeth subequal. Typical sensilla present (not shown in figure).

Remarks. *Entomobrya intermedia* can be easily identified by the presence of two longitudinal stripes, a W-shaped mark on Abd. 4 combined with the presence of Th. 2 macrosetae m_5 and Abd. 3 a_1, and the absence of head macrosetae S_{4i}, Abd. 2 m_{3ep}, and Abd. 3 a_2 (see Table 2 for additional diagnostic characters). Historically, this species was considered a synonym of *E. nivalis*. However, the clear differences in chaetotaxy (see Table 3) and color pattern separate *E. intermedia* from *E. nivalis* and other similar forms such as *E. multifasciata* and female *E. atrocincta*. Further-

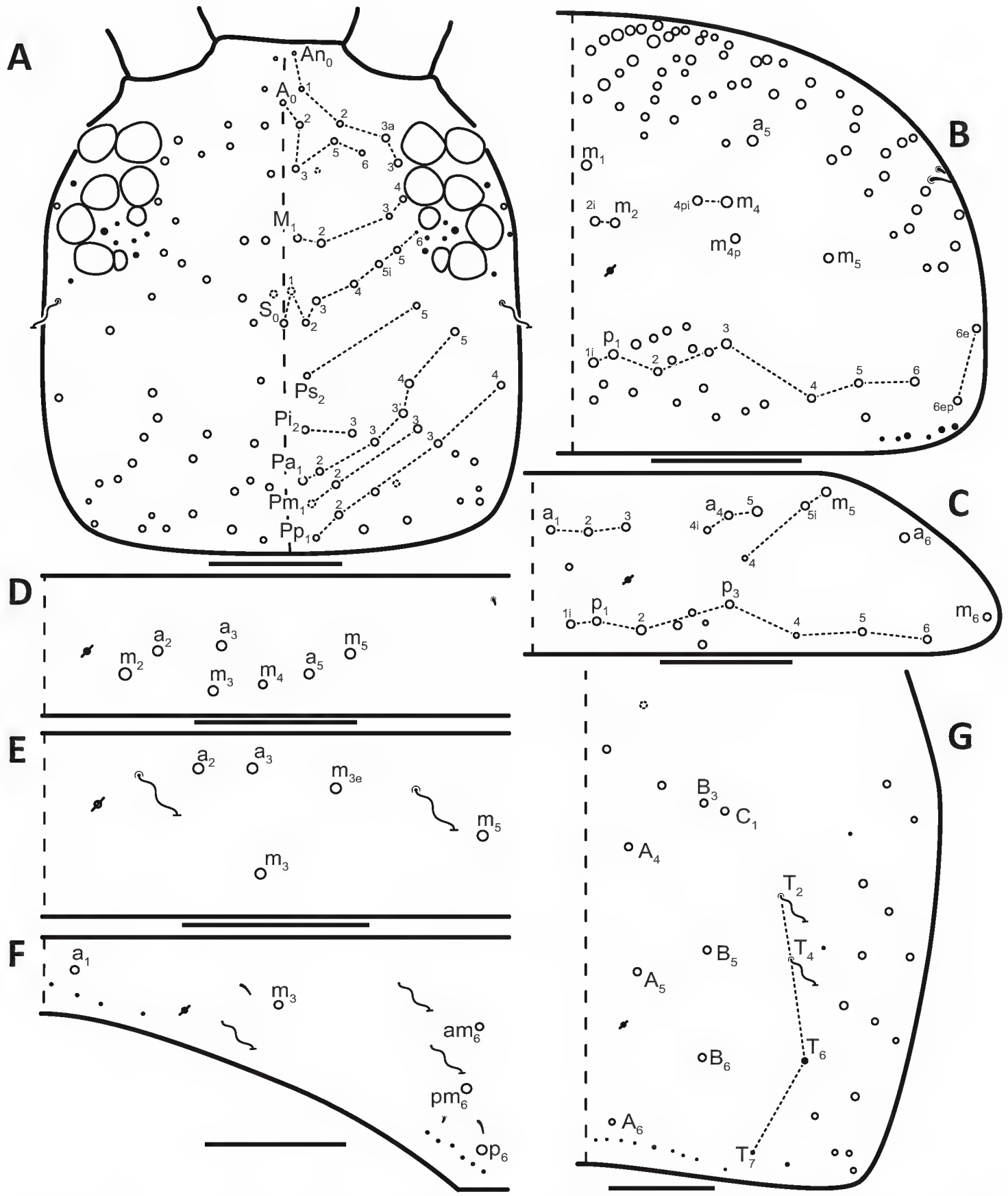


Figure 22. Dorsal chaetotaxy of *Entomobrya intermedia*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

more, molecular data supports the separation of this species from *E. nivalis* (Katz et al. 2015). The combination of chaetotaxy outlined in Table 3 should be used in conjunction with color pattern characters to differentiate this species. Note that observation of chaetotaxy was only examined for specimens collected from Chester County, Pennsylvania. It is likely that specimens from additional localities may re-

veal more variation in chaetotaxy than described here, especially since the chaetotaxy reported in Palearctic specimens (Jordana 2012) is different than those observed in North American specimens.

Distribution. North America and Europe. The actual distribution of *E. intermedia* in North America is unclear, as before Christiansen and Bellinger's (1998) monograph the species was included within *E. nivalis* (Christiansen 1958b). In fact, some records of *E. nivalis*, *E. multifasciata* and female *E. atrocincta*, regardless of date, may actually refer to *E. intermedia*. Suppl. material 1: G shows the distribution of *E. intermedia* in North American as currently understood.

Material examined. USA: 2 on slides, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 23.v.2011, AK11-32; 8 on slides, 12 in vial, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 29.vi.2012, AK12-50.

Entomobrya jubata Katz & Soto-Adames, sp. n.

http://zoobank.org/B178F030-EA9E-43C6-99B3-98C89487F3EB

Figs 16B, 23, 24, 39

Etymology. The word jubatus is Latin for maned, or crested, and refers to the abundance of dorsal macrosetae on the thoracic segments.

Type material. *Holotype*, ♀, USA: Alabama, Covington County, Conecuh National Forest (31.07900,-86.61203), under bark, 2.i.12 (A. Katz & M. DuBray), AK12-9 & AK12-6.

Paratypes, USA: 7 on slides, 20 in vials, Alabama, Covington Co., Conecuh National Forest (31.07900,-86.61203), under bark, 2.i.12 (A. Katz & M. DuBray), AK12-9 & AK12-6.

Description. *Body shape and color pattern.* Body cylindrical, slightly dorso-ventrally flattened. Length up to 2 mm. Color pattern monomorphic (Fig. 23): light brown background with black pigment forming dark transverse bands across the posterior margins of Abd. 4, Abd. 5, and Abd. 6; dark pigment present along lateral margins of Th. 2 through Abd. 2, forming two lateral stripes. Two patches of dark pigment usually present medially on Abd. 4 and may appear to form an incomplete irregular transverse band; Th. 2 entirely white except for black pigment lining anterior and lateral margins; legs range in color from white to light brown to purple near the apex.

Head. Apical bulb of 4th antennal segment usually simple, rarely bilobed. Long differentiated smooth seta on ventral side of 1st antennal segment 3× as long as short setae. Prelabral setae ciliate. Distal margin of the labral papillae with 2-3 seta or spine-like projections. Labial papilla E with lateral appendage almost straight, reaching tip of papilla. Dorsal head chaetotaxy as in Figure 24A: macrosetae An'_0, A_{3a2}, A_{3a3}, M_{3i}, S_6, Ps_3, and Ps_5 always absent; Pi_1 and Pm_{1i} present or absent; S'_0 always present. Eyes G and H small and subequal. Eye patch with 5 setae.

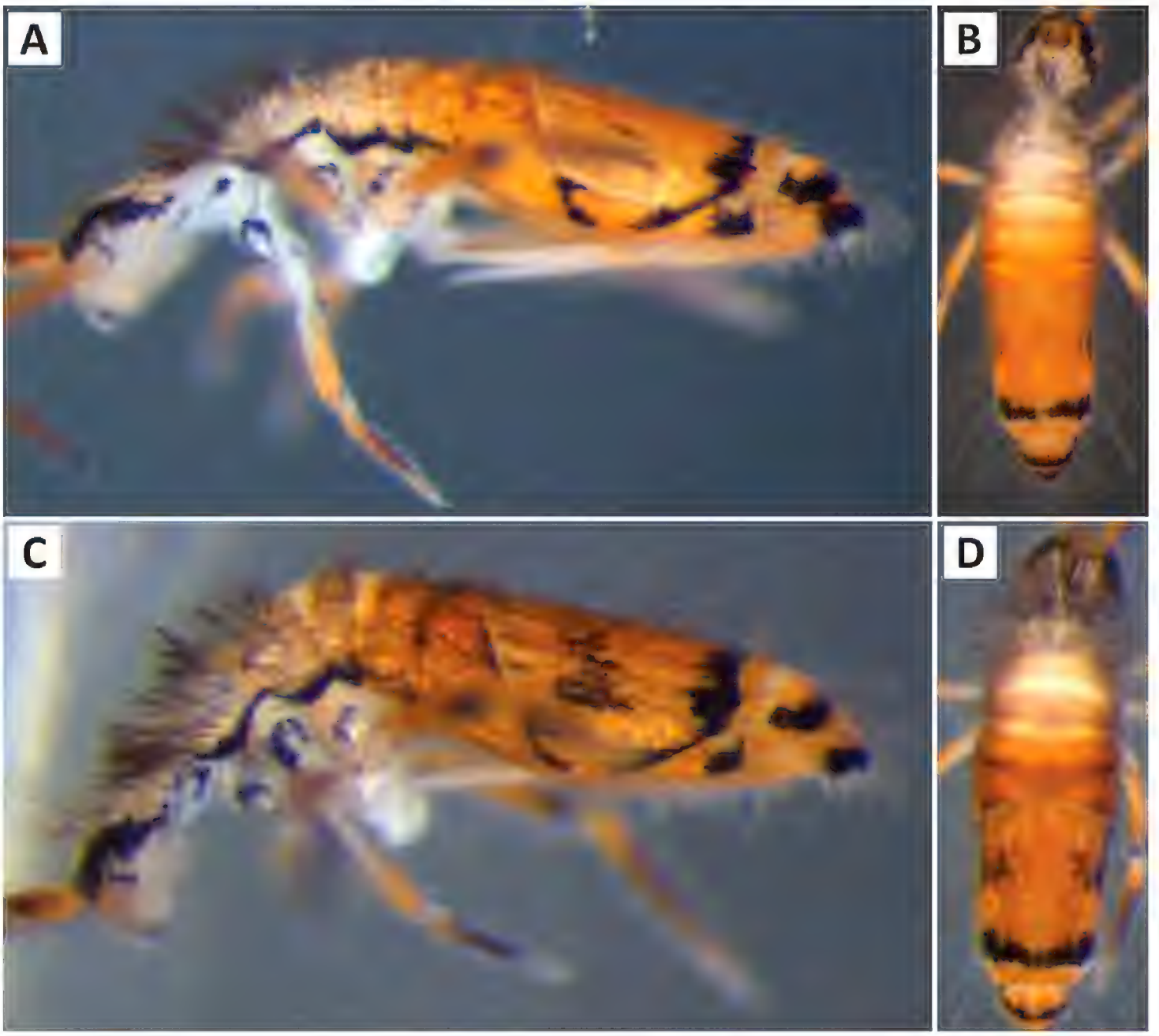


Figure 23. Dorsal color patterns of *Entomobrya jubata* sp. n. collected for this study. Lateral and dorsal views for two individuals (**A, B** and **C, D**) collected from Covington Co., AL.

Thorax. Thoracic chaetotaxy well-developed (Fig. 24B). Th. 2 with all described macrosetae present, except m_{4i3}. Th. 3 macrosetae a_{5e3}, m_4, m_{5p}, and a_7 absent (Fig. 24C). Zone Pm with many supplemental macrosetae present in both thoracic segments.

Legs. Trochanteral organ with triangular setal pattern and up to 25 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length (Fig. 16B).

Abdomen. Abdominal chaetotaxy abundantly developed. Abd. 1 with 12 macrosetae (Fig. 24D). Abd. 2 macrosetae variable: a_2, a_3, m_3, m_{3ep}, m_{3e}, m_{3ei}, m_{3ea}, and m_5 always present; m_{3ep2} and m_{3eai} sometimes present (Fig.24E). Abd. 3 macrosetae a_2, a_3, m_3, am_6, pm_6, and p_6 present; a_1 always absent (Fig. 24F). Abd. 4 chaetotaxy stable, with 7 inner macrosetae (Fig. 24G). Mucronal teeth subequal.

Remarks. *Entomobrya jubata* sp. n. can be easily distinguished by the unique color pattern described above combined with the absence of head macroseta Ps_5, the

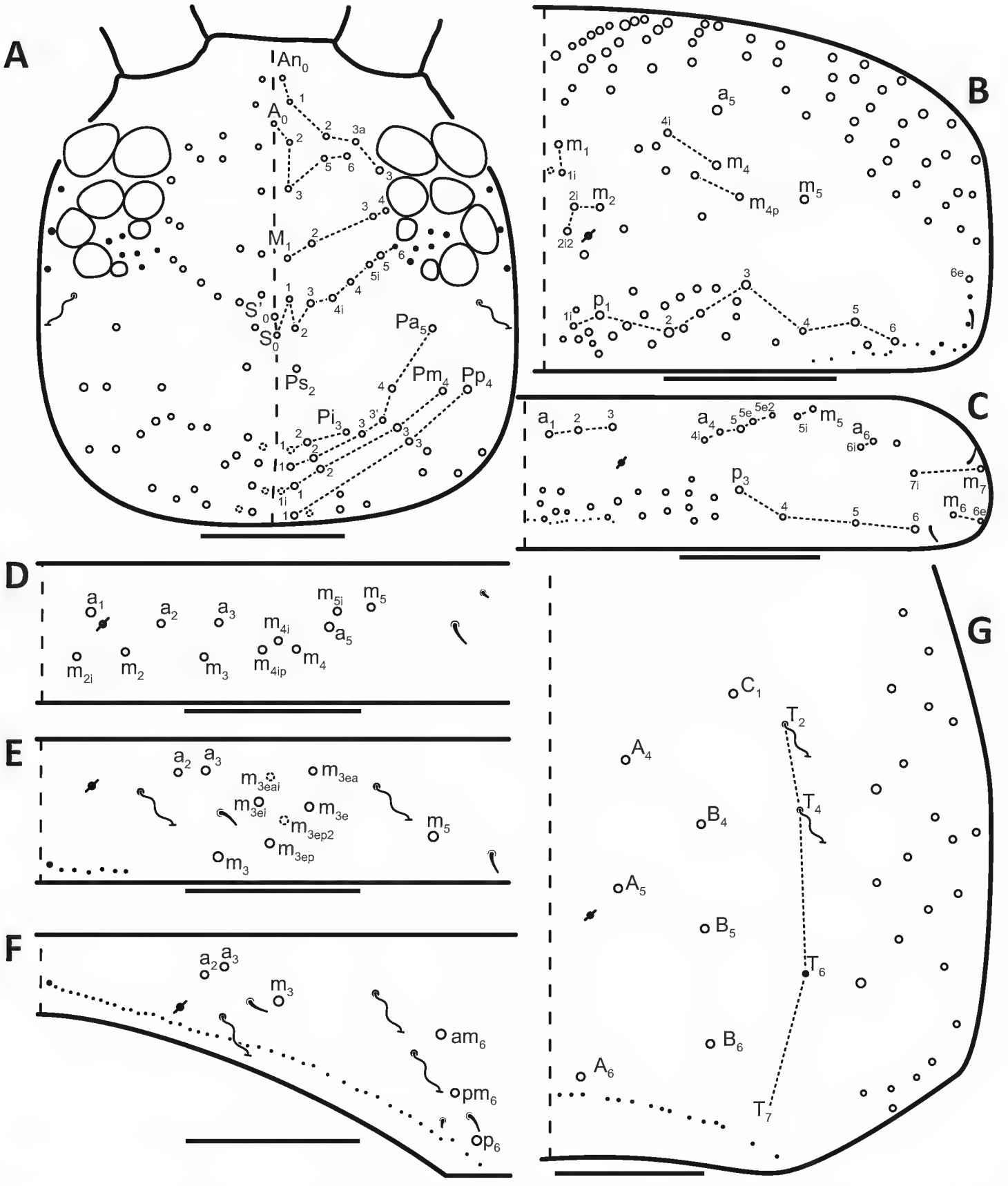


Figure 24. Dorsal chaetotaxy of *Entomobrya jubata* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment (Abd. 2) **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

presence of head macroseta S'_0 and Th. 2 macrosetae m_2 and m_5 (see Table 2 for additional diagnostic characters). This species is closely related to *E. clitellaria* and both share similar chaetotaxy. However, these species can be easily separated by color pattern alone; *E. jubata* sp. n. does not have dark pigment on Th. 3 through Abd.

3. The presence of head macrosetae S'_0 and the absence of head macrosetae Ps_5 also separate *E. jubata* sp. n. from *E. clitellaria*. The color pattern and chaetotaxy exhibited by this species have not been reported in the literature.

Distribution. Endemic to North America. *E. jubata* sp. n. was collected from a single locality in Covington County, Alabama (Suppl. material 2: H).

Entomobrya ligata Folsom, 1924

Figs 25, 26, 39

Description. *Body shape and color pattern.* Body oval and cylindrical. Color pattern stable, monomorphic (Fig. 25), always with four transverse bands; two thin regular bands along the posterior margin of Th. 2 and Th. 3 respectively, an irregular, patchy, thick band covering most of Abd. 3, and a highly irregular and sometimes broken band across the medial section of Abd. 4. A small patch of pigment covers Abd. 5 and Abd. 6. Patterns usually consisting of black or dark blue pigment with a yellow background. Dark pigment usually in patches along lateral margins of Th. 2 through Abd. 4. Antennae with purple pigment, darken near apex. Legs usually white, with small purple patches on apical end of femora.

Head. Apical bulb of 4th antennal segment usually bilobed, sometimes multilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3x short setae. Prelabral setae finely ciliated, seemingly smooth at low magnification under light microscopy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projections. Lateral appendage of labial papilla E slightly curved, relatively thick and short, extending only ¾ papilla length. Dorsal head macrosetae (Fig. 26A) An_{3a2}, An_{3a3}, A_6, M_{3i}, S'_0, S_6, Ps_3, Pi_1, Pm_{1i}, and Pp_2 absent; An'_0, a short mesoseta located medially between both An_0 present. Eyes G and H small and subequal. Eye patch with 3 setae.

Thorax. Th. 2 macrosetae a5, m_1, m_2, m_{2i}, m_4, m_{4p}, and m_{4i} present (Fig. 26B). Th. 3, macrosetae a_1, a_2, a_3, a_4, a_{4i}, a_5, a_{5e}, a_6, a_{6i}, m_5, m_{5i}, m_6, m_{6e}, and m_7 present (Fig. 26C); all posterior macrosetae (series P) present. Zone Pm in both thoracic segments with a moderate number of supplemental macrosetae (Fig. 26B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 17 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

Abdomen. Abdominal chaetotaxy reduced; no macrosetae variation observed. Abd. 1 with 4 macroseta: a_5, m_2, m_3, and m_4 (Fig. 26D). Abd. 2 with 5 macrosetae: a_2, m_3, m_{3e}, m_{3ep}, and m_5 (Fig. 26E). Abd. 3 with 3 macrosetae: m_3, pm_6, and p_6 (Fig. 26F). Abd. 4 with 6 inner macrosetae (Fig. 26G). Mucronal teeth subequal.

Remarks. This species can be identified by the presence of four transverse bands, head mesoseta An'_0 present, four macrosetae on Abd. 1, and only three eye patch setae (see Table 2 for additional diagnostic characters). *Entomobrya unifasciata* sp. n. and *E. neotenica* sp. n. are closely related to this species and form the *E. ligata* complex, char-

Figure 25. Color pattern of *Entomobrya ligata*. Lateral (**A**) and dorsal (**B**) views of specimen collected from Chester Co., PA.

acterized by the presence of only three microsetae in the eye patch, four macrosetae on Abd. 1, and six macrosetae on Abd. 4. *Entomobrya ligata* can be separated from *E. unifasciata* sp. n. and *E. neotenica* sp. n. by characters outlined in Table 6.

E. ligata was described by Folsom (1924) and redescribed by Christiansen (1958b). Both descriptions depict *E. ligata* with four dorsal transverse bands (Folsom described five bands; he considered the pigment on Abd. 5 and 6 an additional band), two of which occur on the posterior margins of the Th. 2 and Th. 3, respectively. Samples were originally diagnosed as *E. ligata* based on chaetotaxy described by Christiansen and Bellinger (1998). However, Katz et al. (2015) showed that a population from Chester Co., Pennsylvania was highly divergent and genetically isolated from other populations. The Pennsylvania population differs from all other populations by the presence of a dark transverse band along the posterior margin of Th. 2 . This dark band is present in all individuals collected in Pennsylvania and absent in individuals from all other localities. The original descriptions by Folsom (1924) and Christiansen (1958b) described *E. ligata* as having this band present and noted a lack of additional variations in color form. Multiple type specimens deposited at the INHS were examined; all collected in the state of New York, and all carry a dark band along the posterior margin of Th. 2 (Suppl. material 3: D–G). Therefore, the combination *E. ligata* is reserved for populations in which individuals carry the posterior band on Th. 2, whereas populations without this band are referred to *E. unifasciata* sp. n. (see below).

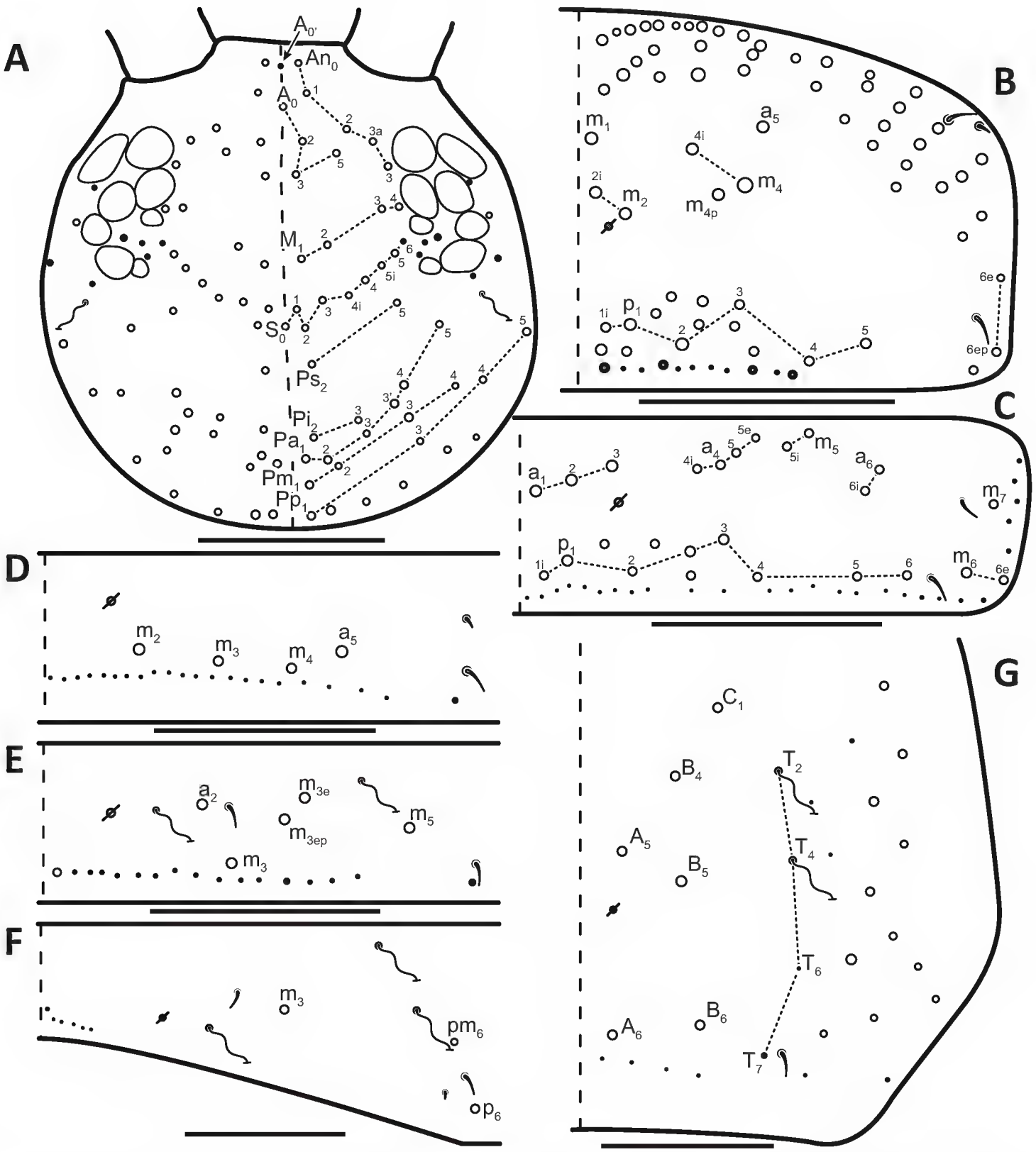


Figure 26. Dorsal chaetotaxy of *Entomobrya ligata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

Distribution. Endemic to North America. The species has been reported as having a wide distribution, occurring east of the Mississippi River to the Atlantic coast (Suppl. material 2: I). However, in light of the new circumscription provided above and the possible confusion with *E. unifasciata* sp. n., most historical reports are questionable, especially those between the western Smokey Mountains and the Mississippi River. The syntypic series of *E. ligata* was collected at different localities in New York State

Table 6. Diagnostic characters to separate species within the ligata complex: *E. ligata*, *E. unifasciata* sp. n., and *E. neotenica* sp. n. Character states: absent (0), present (1).

Species	Head mesoseta An'_0	Abd. 3 macroseta m_{3ep}	1 dark transverse band on posterior margin of Th. 2	2 dark triangular patches on Abd. 3
E. ligata	1	1	1	0
E. unifasciata sp. n.	1	1	0	0
E. neotenica sp. n.	0	0	0(1)[1]	1

[1] Parentheses indicate a rarely observed state.

and all fresh material was collected in Chester Co., Pennsylvania, suggesting the species may be restricted to the northeast section of the country.

Material examined. USA: *Syntypes,* 1 on slide, Karner, N.Y., 7-14-23 (A. Wolf) INHS Cat. No. 528,351; *Cotypes*: 1 in vial, Karner, N.Y., July, 14 1923 (A. Wolf); *Cotypes*: 2 in vial, N.Y., July 8, 1923 (A. Wolf); *Syntypes,* 1 on slide, Mineola, L. I., N.Y., July 8, 1923 (O. W. Barrett) INHS Cat. No. 528,350; *Cotypes*, 1 in vial, Roorhesville, N. Y., Aug 30, 1923 (M. S. Leonard); *Cotypes*: 2 in vial, Roorhesville, N. Y., Aug 30, 1923 (M. S. Leonard). Other material: 1 on slide, 1 in vial, Pennsylvania, Chester Co., Wayne, McKaig Nature Education Center (40.06923,-75.37903), leaf litter, 23.v.2011, AK11-33; 1 on slide Pennsylvania, Chester Co., Wayne, McKaig Nature Education Center (40.06923,-75.37903), bark, 23.v.2011, AK11-34.

Entomobrya multifasciata (Tullberg), 1871

Figs 2, 27, 28, 39

Description. *Body shape and color pattern.* Body oval and cylindrical. One primary color form, with slight variations (Fig. 27); yellow background with black, dark brown or purple pigment forming 5 transverse bands along posterior margins of Th. 2 through Abd. 3. Abd. 4 pattern variable, but usually with 2 triangular patches of pigment along posterior margin of segment that point anteriorly toward a W-shaped mark or broken and irregular transverse band. Abd. 5 and Abd. 6 mostly covered with dark pigment. Dark pigment present along lateral margins of all segments, sometimes in broken patches. Antennae light brown or purple pigment increasingly dark towards the apex.

Head. Apical bulb of 4th antennal segment usually bilobed, sometimes simple. Long differentiated smooth setae on ventral side of 1st antennal segment ≈2–3× short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2-3 seta or spine-like projections (Fig. 2). Labial lateral appendage slightly curved, relatively thin, reaching just beyond tip of papilla. Dorsal head macrosetae (Fig. 28A) An'_0, A_{3a2}, A_{3a3}, M_{3i}, S'_0, S_{4i}, S_6, Ps_3, Pi_1, and Pm_{1i} absent; S_1 and Pm_1 usually present, but may be asymmetrical. Eyes G and H small and subequal. Eye patch with 5 setae.

Thorax. Th. 2 macrosetae a5, m_1, m_2, m_4, and m_{4p} present (Fig. 28B); m_{1i} and m_{2i} sometimes present; posterior macrosetae (series P) present. Th. 3 macrosetae a_1, a_2, a_3,

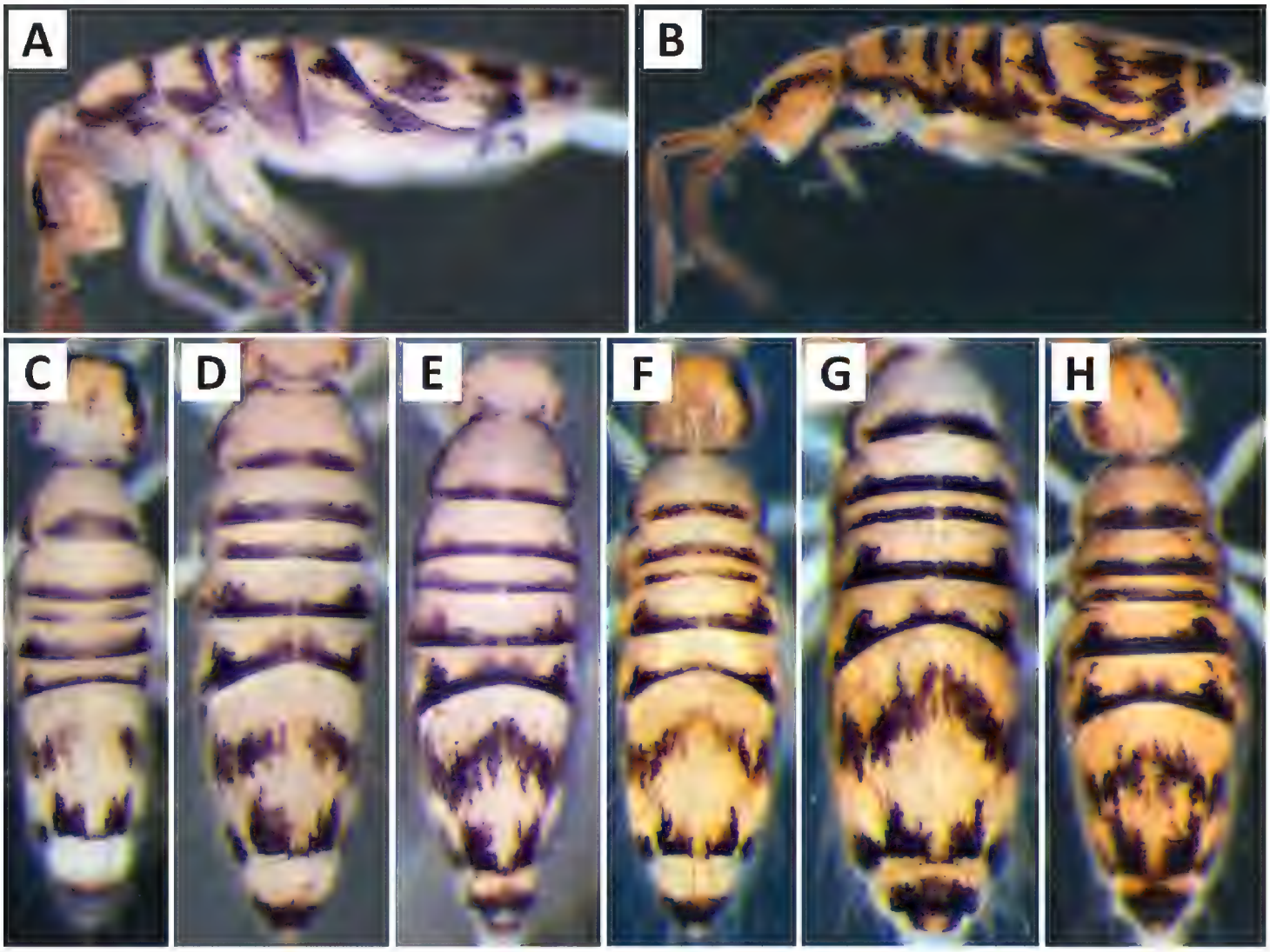


Figure 27. Color patterns of *Entomobrya multifasciata*. All photographed specimens (**A–H**) are from São Miguel Island, Azores, Portugal.

a_4, a_5, a_6, m_5, m_{5i}, m_6, m_{6e}, and m_7 present (Fig. 28C); a_{4i}, a_{5e}, and m_4 sometimes present; posterior macrosetae (series P) present. Zone Pm of both thoracic segments with moderate number of supplemental macrosetae (Figs 3; 39B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 18 setae.

Abdomen. Abd. 1 with 6-8 macrosetae (Fig. 28D). Abd. 2 macroseta a_2, a_3, m_3, m_{3e}, and m_5 are present (Fig. 28E). Abd. 3 macroseta a_1, a_2, a_3, m_3, am_6, pm_6, and p_6 present (Fig. 28F). Abd. 4 with 8-12 inner macroseta (Fig. 28G). Mucronal teeth subequal.

Remarks. *Entomobrya multifasciata* can be distinguished by the color pattern described above combined with the presence of Abd. 3 macrosetae a_1 and a_2 and the absence of macrosetae head S_{4i}, Th. 2 m_5, and Abd. 2 m_{3ep} (see Table 2 for additional diagnostic characters). Chaetotaxy and color pattern observed in this species is almost indistinguishable from that in female *E. atrocincta*. Furthermore, variation in chaetotaxy displayed in *E. atrocincta* obscures most potentially diagnostic characters needed to distinguish *E. multifasciata* from *E. atrocincta*. However, *E. multifasciata* can be recognized by the presence of head macroseta S_{4i} and the morphology of labral papillae; *E. multifasciata* has two to three larger seta or spine-like projections per papillae, while *E. atrocincta* has three to four smaller seta or spine-like projections per papillae (Fig. 2). Table 3 provides additional diagnostic characters separating *E. multifasciata* from

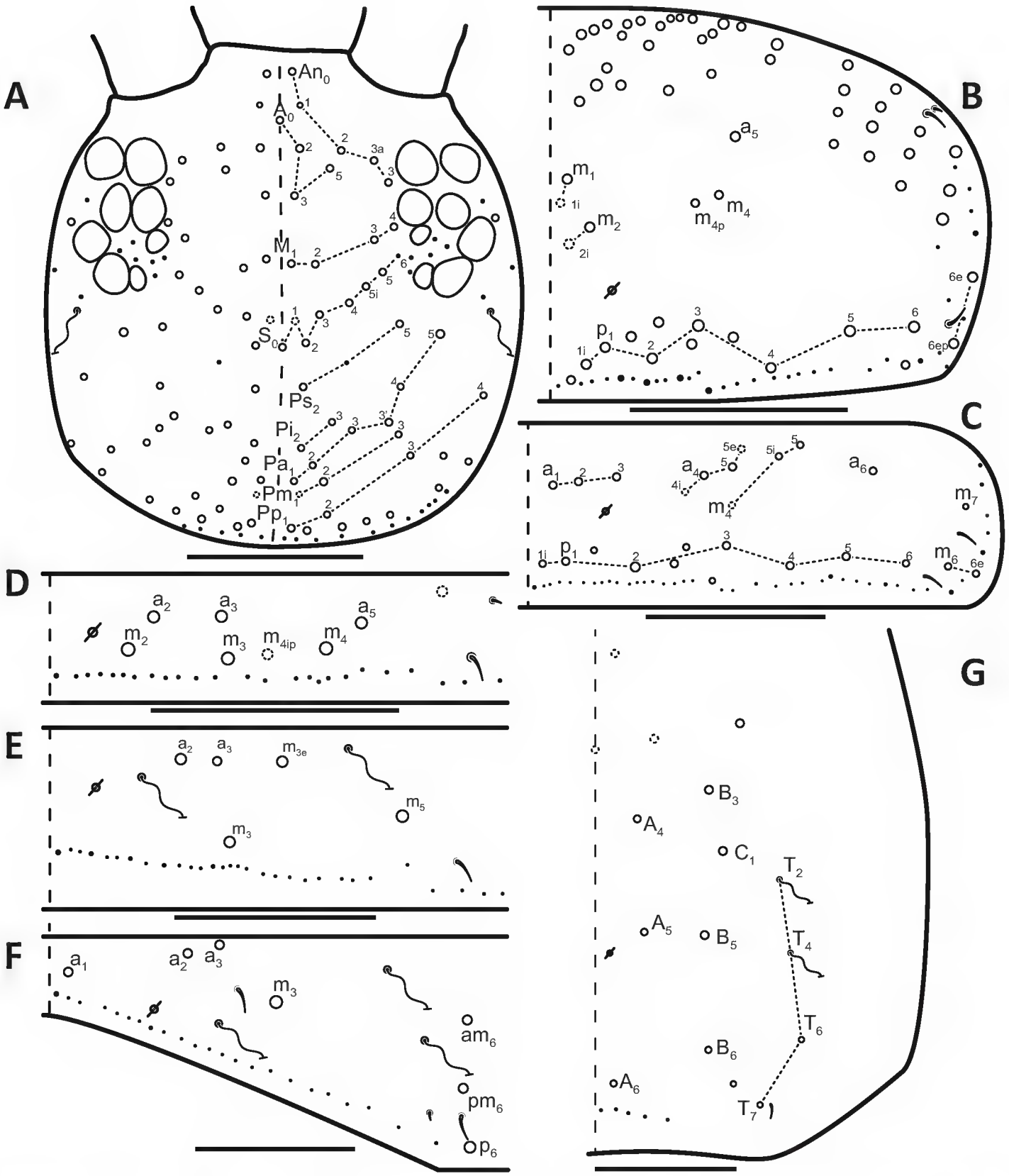


Figure 28. Dorsal chaetotaxy of *Entomobrya multifasciata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

E. nivalis and *E. intermedia*, which share superficially similar color patterns but can be separated by chaetotaxy.

We were unable to obtain North American samples of *E. multifasciata* and the description and diagnosis provided above are based on specimens from São Miguel Island, Azores, Portugal. The Nearctic distribution of this species remains unclear in light of the sexual dimorphism of *E. atrocincta* described in this study (see remarks for *E. atrocincta*). Christiansen and Bellinger (1998) report a widespread distribution,

with localities found across the United States, but they also note that their records may be questionable as a result of likely misidentification. Christiansen and Bellinger (1998) describe the labral papillae of *E. multifasciata* as having three to four small seta or spine-like projections, a condition that resembles those in *E. atrocincta*, possibly indicating an identification error. In the Collembola of North America, Christiansen and Bellinger (1998) report a male genital plate, presumably from an individual with the *E. multifasciata* pattern collected in Massachusetts, which eliminates the possibility that the specimen could have been a female *E. atrocincta*. Since a number of samples were collected in the Northeastern United States (Christiansen and Bellinger 1998), the presence of *E. multifasciata* in North America cannot be ruled out. However, in the course of the present study, all specimens collected bearing the banded color pattern were female *E. atrocincta* and were usually accompanied by male *E. atrocincta*.

Distribution. North America (Christiansen and Bellinger 1998), Hawaii (Christiansen and Bellinger 1992), Europe and Russia (Jordana 2012). Records from North America and Hawaii are questionable due to likely misidentification of *E. atrocincta* females. See Suppl. material 2: J for a distribution map and below for a list of material examined with collection and locality information.

Material examined. PORTUGAL: 2 on slides, 10 in vial, Azores, São Miguel Island, (J. Marcelino), vial #119; 6 on slides, 10 in vial, Azores, São Miguel Island, (J. Marcelino), vial #86; 5 in vial, Azores, São Miguel Island, (J. Marcelino), vial #55; 4 in vial, Azores, Terceira Island, (J. Marcelino), vial #9; 4 in vial, Madeira Island, (J. Marcelino), vial #2.

***Entomobrya neotenica* Katz & Soto-Adames, sp. n.**
http://zoobank.org/C70B2A90-547A-4A0F-9A4E-E5F33B83BF8A
Figs 2, 16C, 29, 30, 39

Etymology. This species is named for its apparent neoteny; small size and reduced chaetotaxy.

Type material. *Holotype*, ♂, USA: Alabama, Lawrence County, William B. Bankhead National Forest (34.3369,-87.3461), leaf litter, 9.viii.2011, AK11-112.

Paratypes, USA: 1 on slide, 1 in vial, Alabama, Clay Co., Talladega National Forest (33.19723,-86.06325), moist leaf litter, 2.i.12 (A. Katz & M. DuBray), AK12-2; 1 in vial, Alabama, Lawrence Co., William B. Bankhead National Forest (34.3369,-87.3461), leaf litter, 9.viii.2011, AK11-112; 1 on slide, Florida, Taylor Co., Econfina River State Park (30.0656,-83.9107), under bark, 9.viii.2011, AK11-116; 2 on slides, 18 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, moist leaf litter under bark on fallen tree, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 1 on slide, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area (36.5354,-87.9214), forest floor leaf litter, 3.v.2011, AK-43.

Description. *Body shape and color pattern*. Body oval and cylindrical. Length up to 1.15 mm. One primary color form (Fig. 29): white and yellow or orange background

Figure 29. Color pattern of *Entomobrya neotenica* sp. n. Lateral and dorsal views of specimens collected from Lawrence Co., AL (**A** and **D**) and Clay Co., AL (**B** and **C**).

with black or dark purple pigments forming two lateral triangles on the sides Abd. 3; triangles sometimes reduced to irregular patches. Additional irregular patches of pigment usually on lateral margins of all segments. A band sometimes present along posterior margin of Th. 2. Abd. 5 with 2 dark spots, sometimes forming irregular triangular shapes. Antennae usually light purple near apex and relatively long. Legs usually white, with small purple patches on apical end of femora.

Head. Apical bulb of 4th antennal segment simple or bilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈2–2.5× short setae. Four prelabral setae finely ciliate, appearing smooth under light microscopy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projections (Fig. 2). Labial lateral appendage slightly curved, relatively thick, not reaching tip of papilla. Dorsal head chaetotaxy reduced (Fig. 30A), macrosetae An'_0, An_{3a2}, An_{3a3}, A_6, M_{3i}, S'_0, Ps_3, Pi_1, Pi_2, Pm_1, Pm_{1i}, and Pp_2 absent; S_{5i} usually present; S_{4i} and S_6 usually absent. Eyes G and H smaller than A-F but enlarged; G slightly larger than H. Eye patch with 3 setae.

Thorax. Thoracic chaetotaxy reduced, with relatively few supplementary setae. Th. 2 macrosetae a5, m_1, m_2, m_4, m_{4p}, and m_{4i} present (Fig. 30B); all posterior macrosetae (series P) present. Th. 3 macrosetae a_1, a_2, a_3, a_4, a_{4i}, a_5, a_{5e}, a_6, a_{6i}, m_5, m_{5i}, m_6, m_{6e}, and m_7 present (Fig. 30C). Both thoracic segments with few supplemental macrosetae present in zone Pm (Fig. 30B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 20 setae. Unguis with 4 inner teeth (Fig. 16C). Unguiculus acuminate with small serrations on inner edge.

Abdomen. Abdominal chaetotaxy reduced; no macrosetae variation observed. Abd. 1 with 4 macrosetae; a_5, m_2, m_3, and m_4 (Fig. 30D). Abd. 2 with 4 macrosetae: a_2, m_3,

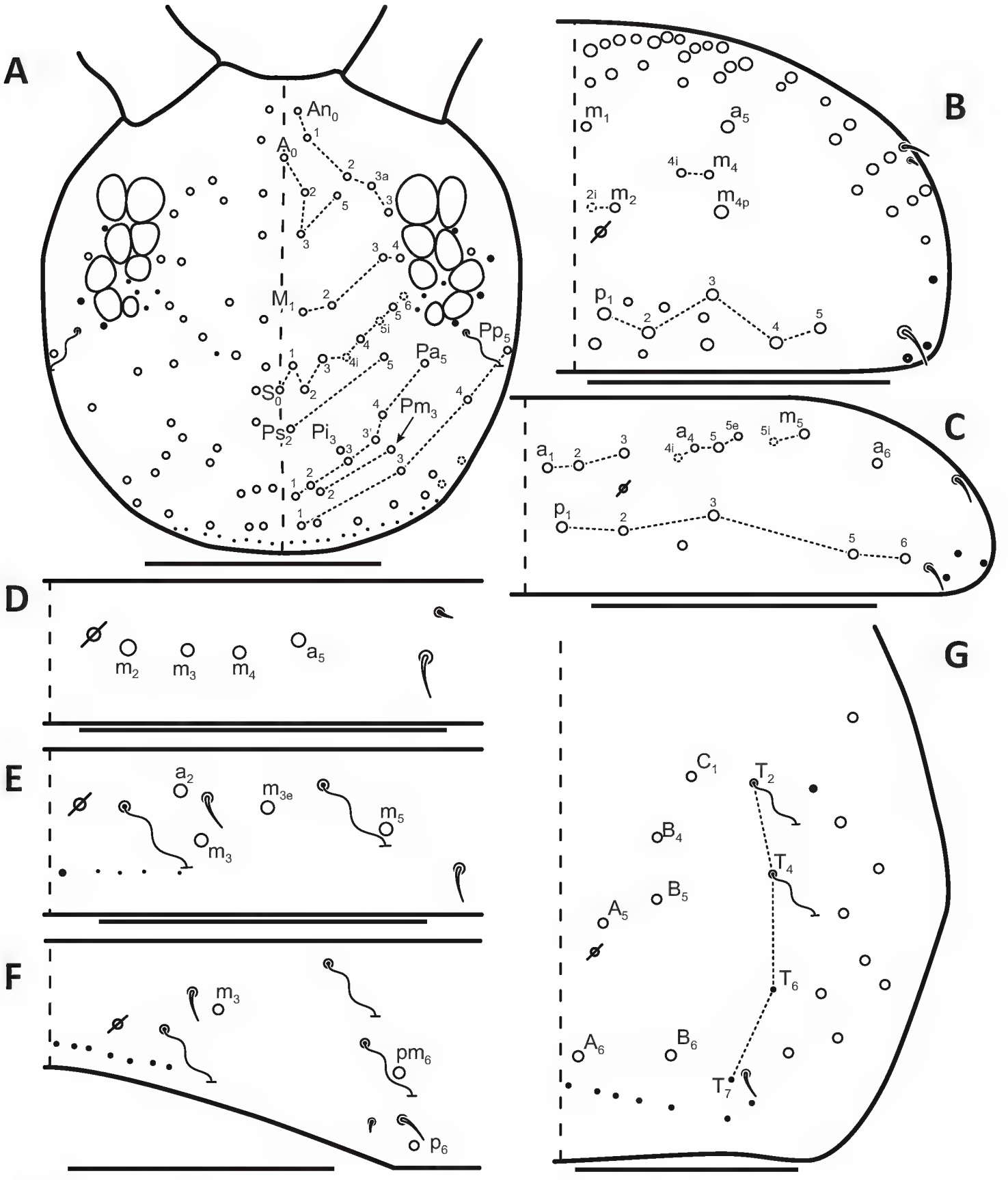


Figure 30. Dorsal chaetotaxy of *Entomobrya neotenica* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

m_{3e}, and m_5 (Fig. 30E). Abd. 3 with 3 macrosetae: m_3, pm_6, and p_6 (Fig. 30F). Abd. 4 with 6 inner macrosetae (Fig. 30G). Mucronal teeth subequal; mucronal spine smooth.

Remarks. *Entomobrya neotenica* sp. n. can be diagnosed by the presence of two lateral dark triangular shaped or irregular spots on Abd. 3, only 3 setae in eye patch, and the absence of head mesoseta An'_0 and Abd. 3 macroseta m_{3ep} (see Table 2 for ad-

ditional diagnostic characters). This species is included in the *E. ligata* complex (see remarks for *E. ligata*) and has a unique color pattern and chaetotaxy never before reported in the literature. *E. neotenica* sp. n. is exceptionally small compared to most Nearctic *Entomobrya*. In fact, most individuals were thought to be juvenile forms of *E. ligata* prior to the observation of the male genital plate. *Entomobrya neotenica* sp. n. is very similar to *E. ligata* and *E. unifasciata* sp. n., but can be separated by characters outlined in Table 6.

Distribution. Endemic to North America (Suppl. material 2: K)

Entomobrya nivalis (Linnaeus), 1758

Figs 2, 31, 32, 39

Description. *Body shape and color pattern*. Body cylindrical. One primary, but variable, color form (Fig. 31): yellow or white background with black, dark brown or purple pigment always forming thin transverse bands along the posterior margin of Th. 3, and Abd. 2 through Abd. 6. Additional transverse bands present or absent on Th. 2 and Abd. 1. Abd. 4 usually with U-shaped or "11"-shaped pattern connecting basally with band along posterior margin. Antennae usually lack dark pigmentation, sometimes with light brown or purple pigment, darkening near the apex.

Head. Apical bulb of 4th antennal segment usually bi- or trilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Ornamentation of the distal margin of the labral papillae with 3-4 small seta or spine-like projections per papilla (Fig. 2). Labial lateral appendage slightly curved, relatively thick, nearly reaching tip of papilla. Labial triangle chaetotaxy normal, one specimen with a supplemental ciliate seta internal to M1 (one side only). Dorsal head chaetotaxy (Fig. 32A) with macrosetae An'_0, A_{3a2}, A_{3a3}, M_{3i}, S'_0, Ps_3, Pi_2, Pi_3, Pm_{1i}, and Pm_2 absent; S_1 usually present, A_6, S_6, Ps_3, and Pa_2 sometimes present. Eyes G and H small and subequal. Eye patch with 5 or 6 setae.

Legs. Trochanteral organ with triangular setal pattern and up to 34 setae. Unguis with 4 internal teeth; basal teeth located approximately 60% of inner claw length. Unguiculus acuminate with small serrations on internal edge.

Thorax. Th. 2 macrosetae a5, m_1, m_2, m_4, m_{4i}, m_{4pi}, and m_5 present (Fig. 32B); m_{2i} and m_{2i2} usually present; m_{1i} and m_{4p} sometimes present; all posterior macrosetae (series P) are present. Th. 3 macrosetae a_1, a_2, a_3, a_4, a_{4i}, a_5, a_6, a_7, m_5, m_{5i}, m_6, and m_7 present (Fig. 32C); most posterior macrosetae (series P) present, p3 present or absent. Both thoracic segments with a moderate number of supplemental macrosetae in zone Pm (Fig. 32B,C).

Abdomen. Abd. 1 with 7-10 macrosetae (Fig. 32D). Abd. 2 macroseta a_2, a_3, m_3, m_{3e}, m_{3ep}, and m_5 present; m_{3ei} and m_{3ea} usually absent (Fig. 32E). Abd. 3 macroseta a_1, m_3, am_6, pm_6, and p_6 present (Fig. 32F); a_3 usually absent. Abd. 4 with 7-8 inner macrosetae (Fig. 32G). Mucronal teeth subequal.

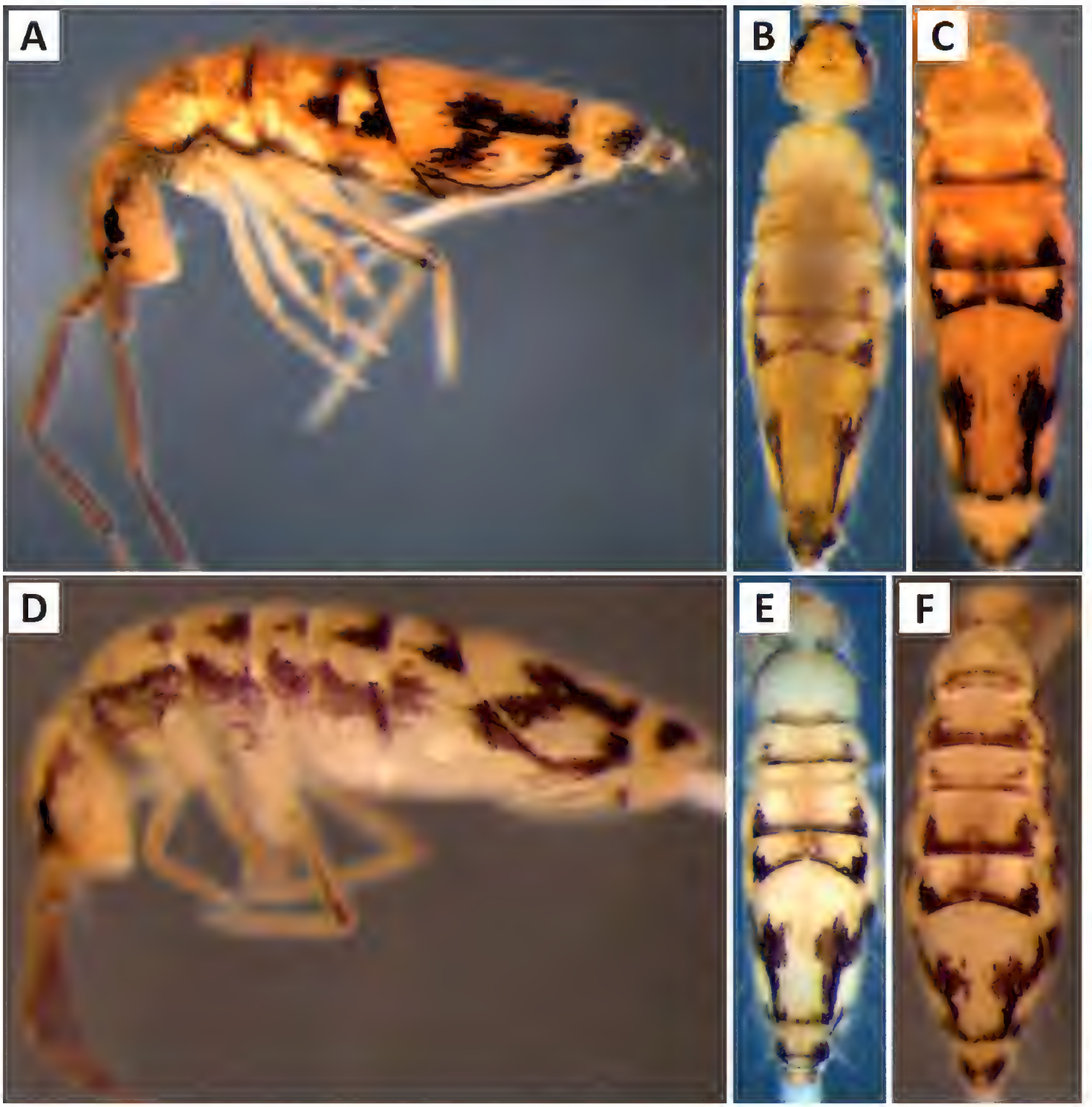


Figure 31. Color pattern of *Entomobrya nivalis* collected from: **A** Lamoille Co., VT **B** Sauk Co., WI **C** Hancock Co., ME **D** Lamoille Co., VT **E** Hancock Co., ME **F** Sauk Co., WI.

Remarks. *Entomobrya nivalis* can be diagnosed by the presence of a U-shaped or "11" shaped pattern on Abd. 4 combined with the presence of macrosetae head S_{4i}, Th. 2 m_5, Abd. 2 m_{3ep}, and Abd. 3 a_1, and the absence of Abd. 3 a_2 (see Table 2 for additional diagnostic characters). This species has a highly variable color pattern with many intermediate forms that intergrade with *E. atrocincta* females, *E. intermedia*, and *E. multifasciata*. However, *E. nivalis* can be separated from these species by chaetotaxy (Table 3) and, with careful consideration, color pattern; the presence of a U-shaped or "11" shaped pattern on Abd. 4 is unique to this species. Therefore, it is critical to evaluate chaetotaxy in addition to color pattern when making a species diagnosis.

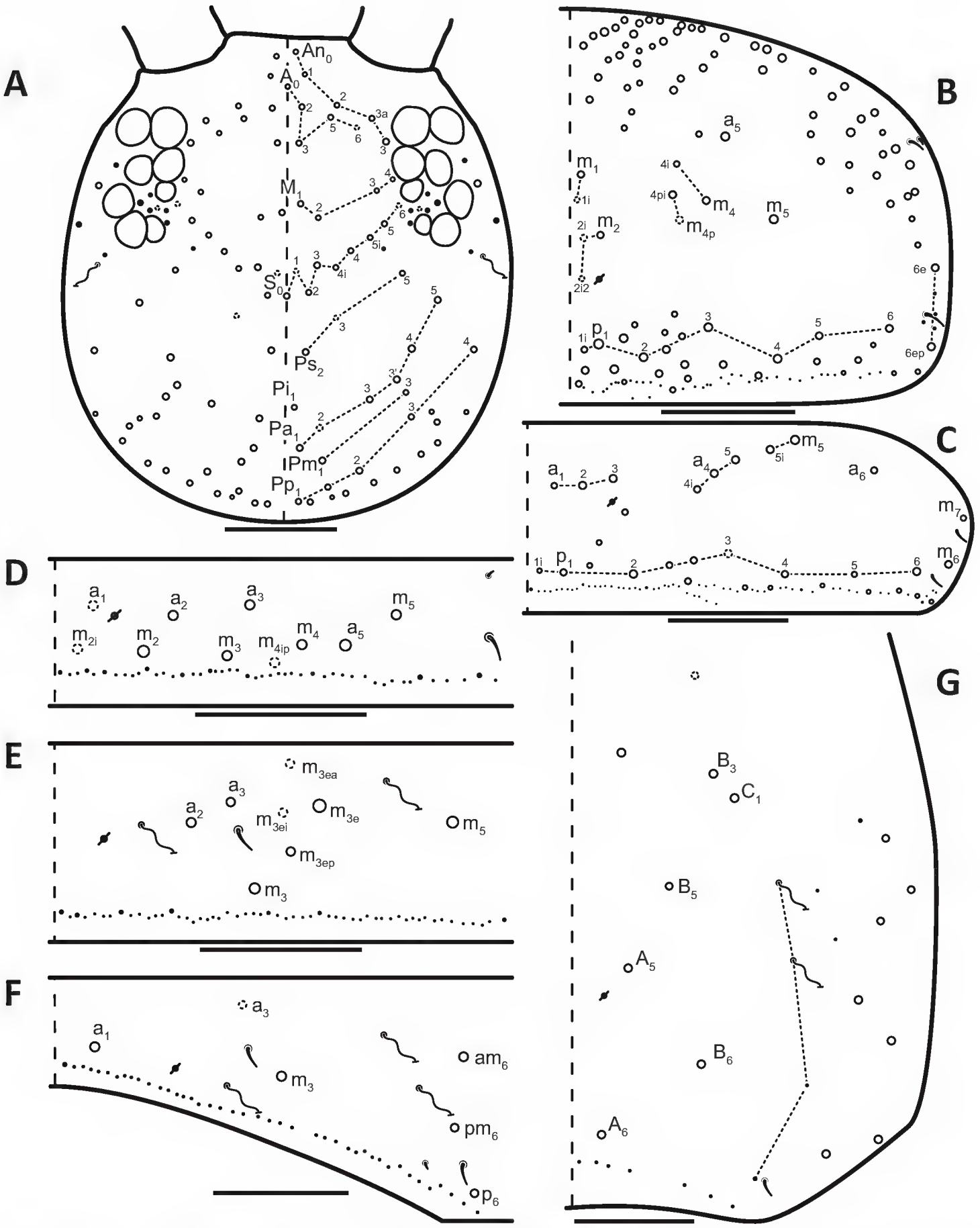


Figure 32. Dorsal chaetotaxy of *Entomobrya nivalis*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

It is important to note that the large genetic distances between presumably conspecific individuals (Katz et al. 2015; Feng Zhang, personal communication), differences in chaetotaxy between populations in North America and Europe (Jordana 2012), and

variable color pattern among populations across its world-wide distribution, suggests that *E. nivalis* likely represents a cryptic species complex.

Distribution. North America and Europe. Records of *E. nivalis* in North America are suspect if diagnosed without considering chaetotaxy given the superficial similarities in color form expressed by *E. atrocincta* females (See Fig. 9G,H). See Suppl. material 2: L for a distribution map and below for a list of material examined with collection and locality information.

Material examined. USA: 2 on slides, 50+ in vial, Maine, Hancock Co., Acadia National Park (44.353823,-68.224754), moss, veg. sweep (blueberry, juniper, populus), 17.viii.2011 (E. C. Bernard), #2009-37; 3 on slides, 5 in vial, Pennsylvania, Allegheny Co., Allegheny National Forest, Dewdrop campground (41.83092,-7895937), leaf litter, 8.vii.2008 (S. M. Shreve); 1 on slide, Vermont, Chittenden Co., Red Rock, Locality I (44.44493,-73.23040), leaf litter, 6.vi.2011 (J. Fisher); 2 on slides, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 13.vi.2001 (J. Fisher); 1 on slide, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 4.vii.2001 (J. Fisher); 2 on slides, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 26.vii.2001 (J. Fisher); 1 on slide, Vermont, Lamoille Co., Stowe (44.48377,-72.69859), leaf litter, 24.vii.2001 (J. Fisher); 1 on slide, Vermont, Lamoille Co. (44.54858,-72.79393), DNA ID#: 12-FSVTlam-ni-1; 1 on slide, Vermont, Washington Co., Locality II, Barre (44.19968,-72.50135), leaf litter, 8.vi.2001 (J. Fisher); 2 on slides, Vermont, Washington Co., Locality II, Barre (44.19968,-72.50135), leaf litter, 24.vii.2001 (J. Fisher); 2 on slides, 6 in vial, Vermont, Rutland Co., Green Mountain National Forest, Greendale Recreation Area (43.35112,-72.82225), leaf litter, 10.vii.2008 (S. M. Shreve); 1 on slide, 6 in vial, Wisconsin, Dodge Co., Horicon Marsh National Wildlife Refuge, end of Dike Rd (43.52736,-88.64381), 12.vi.2011, AK11-47; 2 on slides, 7 in vial, Wisconsin, Sauk Co., Devil's Lake State Park, 0.5mi down Steinke Basin Loop trail (43.4255,-89.71039), 12.vi.2011, AK11-50.

Entomobrya quadrilineata Bueker, 1939

Figs 2, 13C, 33, 34, 39

Description. *Body shape and color pattern.* Body very elongate and cylindrical. Color pattern monomorphic (Fig. 33); white or yellow background with black or dark blue or purple pigment forming two dark parallel longitudinal stripes extending from anterior margin of Th. 2 through posterior margin of Abd. 2. Dark pigment present along lateral margins of Th. 2 through Abd. 2, forming 2 additional lateral longitudinal bands. Abd. 3 and Abd. 4. with 2 angled bands. A small patch of dark pigment sometimes occurs medially on Abd. 3. An irregular and (and sometimes incomplete) transverse band present along posterior margin of Abd. 4. Abd. 5 with 2 small lateral patches of pigment sometimes forming 2 triangles. Abd. 6 usually pale, without dark pigment. Apex of femora usually with a patch of dark pigmentation. Antennae usually

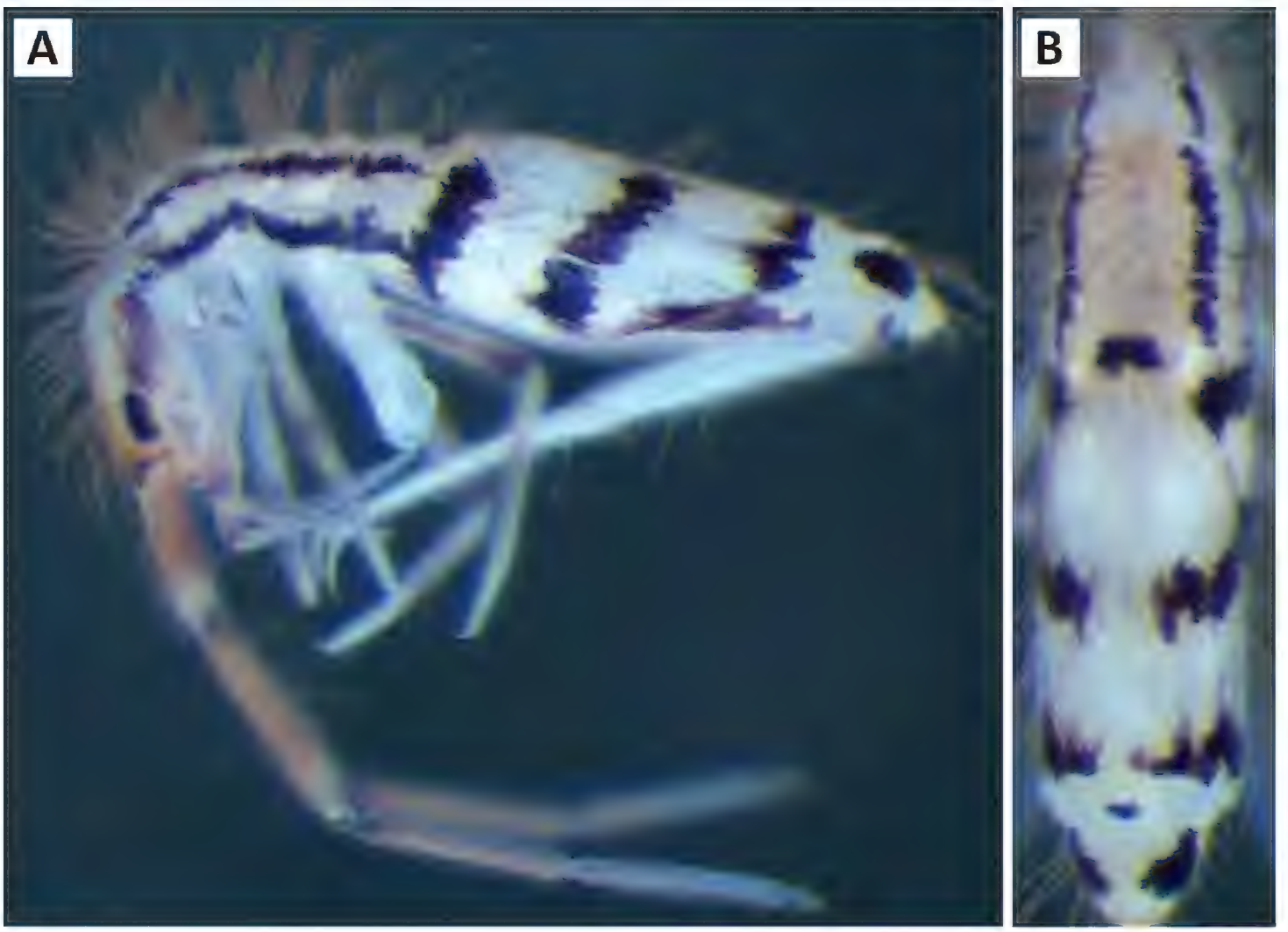


Figure 33. Color pattern of *Entomobrya quadrilineata*. Lateral (**A**) and dorsal (**B**) views of specimen collected from Stewart Co., TN.

with some light brown or purple pigment, darkening near apex. Longitudinal bands usually present in juveniles.

Head. Apical bulb of 4th antennal segment usually bilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈4× short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E almost twice as long as papilla. Labial triangle chaetotaxy slightly irregular and atypical: M1, r, E, L1, L2, all ciliate; r significantly smaller than other setae; a supplementary ciliate seta sometimes present internal to M1, and relatively difficult to observe; A1-A5 smooth. Dorsal head chaetotaxy (Fig. 34A) with macrosetae An'_0, An_{3a3}, A_6, S'_0, S_6, Ps_3, and Pm_{1i} absent; Pi_1 sometimes present. Eyes G and H small and subequal. Eye patch with 5 or 6 setae.

Thorax. Thoracic chaetotaxy greatly developed, with high levels of variation and many supplemental macrosetae. Th. 2 zone A enlarged and sometimes merging with medial macrosetae forming a single, large patch of setae (Fig. 34B). Th. 3 macrosetae m_4, m_{5p}, a_{6i}, and a_7 absent (Fig. 34C). Both thoracic segments with zone Pm enlarged, with many supplemental macrosetae present forming wing-like patches and extending near anterior row (Fig. 34B,C). Th. 3 pseudopore displaced anteriorly, very close to macrosetae a_1, a_2, and a_3.

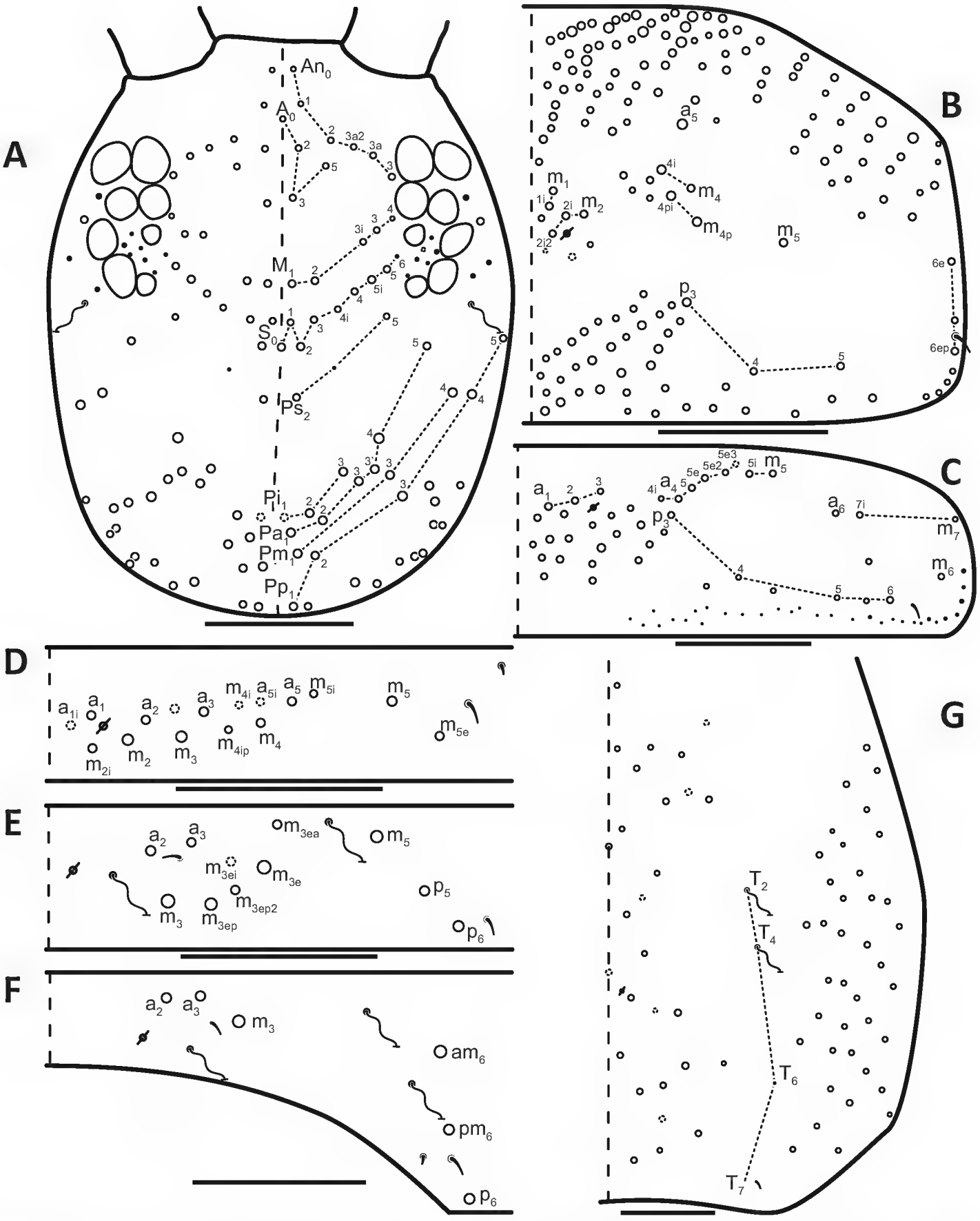


Figure 34. Dorsal chaetotaxy of *Entomobrya quadrilineata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

Legs. Trochanteral organ with rectangular setal pattern and up to 41 setae.

Abdomen. Abdominal chaetotaxy highly developed. Row of microsetae along posterior margin present in all segments (not displayed in figure). Abd. 1 with 12-16 macrosetae (Fig. 34D). Abd. 2 macrosetae a_2, a_3, m_3, m_{3e}, m_{3ep}, m_{3ep2}, m_{3ea}, m_5, p_5, and

p_6 present (Fig. 34E); m_{3ei} usually present. Abd. 3 macrosetae a_2, a_3, m_3, am_6, pm_6, and p_6 present (Fig. 34F); a_1 absent. Abd. 4 elongate, with at least 25 macrosetae internal to bothriotricha T_2 and T_4 (Fig. 34G), but number of macrosetae extremely variable between individuals and even within single individuals (Fig. 16C). Pseudopores on Abd. 4 with asymmetric relative insertions with respect to macroseta and bothriotricha (Fig. 13C). Mucronal teeth subequal

Remarks. *Entomobrya quadrilineata* can be identified by the presence of 2 parallel longitudinal thoracic stripes combined with the presence of head macrosetae M_{3i} and ps_5 and the absence of Abd. 3 macroseta a_1 (see Table 2 for additional diagnostic characters). This species is part of the *E. bicolor* complex. Many supplemental macrosetae and extreme setal variation make it difficult to separate species in this group using chaetotaxy and other traditional morphological characters. However, there are clear differences in color pattern which can be used to differentiate *E. quadrilineata* from *E. decemfasciata* and *E. bicolor* (Table 5); *E. quadrilineata* has a relatively stable and easily distinguishable color pattern and can be diagnosed by the presence of two parallel longitudinal stripes extending from the anterior margin of Th. 2 through the posterior margin of Abd. 2 (Fig. 33). See remarks for *E. decemfasciata* for more information regarding species diagnosis and delimitation. Also, see Suppl. material 3: A,B for photographs of the type specimen.

Distribution. Endemic to North America (Suppl. material 2: M). Many records may be misidentifications due to similarity in color pattern and chaetotaxy with *E. decemfasciata*. Records of *E. quadrilineata* without thoracic stripes are most likely *E. decemfasciata*.

Material examined. USA: *Neo-holotype* (Christiansen 1951), 1 on slide, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr, INHS Cat. No. 529,188; *Neo-paratype* (Christiansen 1951), 1 on slide, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr, INHS Cat. No. 529,189; *Neo-paratype* (Christiansen 1951), 1 in vial, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr; 4 on slides, 13 in vial, Illinois, Monroe Co., Kidd Lake Marsh State Natural Area (37.97211,-89.80135), leaf litter #42, 29.ix.2009 (S. Taylor & F. Soto-Adames), sjt09-114; 1 in vial, Illinois, Pope Co., Bell Smith Springs (37.51882,-88.65782), bare sandstone bedrock above canyon in mosses and lichens, 28.viii.2011 (J. Cech); 1 on slide, Illinois, Vermilion Co., Kennekuk Cove County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), vacuum hill prairie, 16.vi.2011, AK11-59a; 1 in vial, Illinois, Wayne Co., County Road 580N (38.34122,-88.23992), leaf litter from north side of road, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-61; 4 in vial, Illinois, Wayne Co. (38.32500,-88.25016), 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-63; 1 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, .25mi down Neville Bay Rd. (36.60757,-87.93457), leaf litter on forest floor, 31.v.2011, AK11-44; 1 on slide, 2 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-105.

***Entomobrya unifasciata* Katz & Soto-Adames, sp. n.**
http://zoobank.org/A68AD8F0-1545-471C-812D-2F066A08858F
Figs 2, 16D, 35, 36, 39

Etymology. From the Latin words *uno* and *fasciatus*, which translates to "one band". This species has only one band found along the posterior margin of the metathorax, a character that distinguishes it from *E. ligata*, which has two bands; one along each posterior margin of the meta- and mesothorax.

Type material. *Holotype*, ♂, USA: Kentucky, Laurel County, Levi Jackson State Park (37.08247,-84.04528), leaf litter collected at night, 28.v.2011, AK11-37.

Paratypes, USA: 2 on slides, 1 in vial, Georgia, Union Co., Brasstown Bald Rd., tiny water trickle near road surrounded by dryish leaves (34.86040,-83.80193), leaf litter, 26.v.2011 (E. C. Bernard) #2011-28; 1 on slide, Kentucky, Laurel Co., Levi Jackson State Park (37.08247,-84.04528), leaf litter collected at night, 28.v.2011, AK11-37; 1 on slide, 2 in vial, North Carolina, Henderson Co., Blue Ridge Parkway, Mill River Overlook (35.4482,-82.71963), under bark on logs, 4.vi.2007 (E. C. Bernard), 07031EB; 10 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), leaf litter along road, 29.v.2011, AK11-38; 1 on slide, 35 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), under bark, 29.v.2011, AK11-39;11 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), leaf litter by river, 29.v.2011, AK11-40; 1 on slide, 1 in vial, Tennessee, Sevier Co., Great Smoky Mountains National Park, 1 mi down greenbrier Rd. (35.72640,-83.40173), leaf litter by stream, 30.v.2011, AK11-41; 1 in vial, Tennessee, Sevier Co., Great Smoky Mountains National Park, 1 mi down greenbrier Rd. (35.72640,-83.40173), leaf litter stuck in nook of tree, 30.v.2011, AK11-42.

Description. *Body shape and color pattern*. Body oval and cylindrical. Length up to 1.85 mm. Color pattern stable (Fig. 35), always with 3 transverse bands, 1 thin regular band along posterior margin of Th. 3, an irregular, patchy, thick band covering most of Abd. 3, and a highly irregular and sometimes broken band across medial section of Abd. 4. A small patch of pigment covers Abd. 5 and 6. Patterns usually consisting of black or dark blue pigment on a yellow background. Dark pigment usually occurring in patches along lateral margins of Th. 2 through Abd. 4. Small, rectangular black patches may occur in pairs on posterior margin of Th. 2, Abd. 1, and Abd. 2. A faint transverse band sometimes on posterior margin of Th. 2, but if present, always much lower in opacity compared to transverse band along posterior margin of Th. 3. Antennae with purple pigment, darker near apex. Legs usually white, with small purple patches on apical end of femora. A medial ring of purple pigment also occurs on tibiotarsus of hind legs.

Head. Apical bulb of 4th antennal segment usually bilobed, sometimes simple. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Four prelabral setae finely ciliate, seemingly smooth at low magnification under light micros-

Figure 35. Color pattern of *Entomobrya unifasciata* sp. n. collected from: **A** Sevier Co., TN **B** Union Co, GA **C** Laurel Co., KY.

copy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E short, extending only ¾ papilla length. Dorsal head chaetotaxy (Fig. 36A) with macrosetae A_6, M_{3i}, S_6, Ps_3, Pi_1, Pm_{1i}, and Pp_2, absent; S'_0 usually absent, but observed in 2 individuals; An'_0, a short mesoseta present medially between both An_0. Eyes G and H small and subequal. Eye patch with 3 setae.

Thorax. Th. 2 macrosetae a5, m_1, m_2, m_4, m_{4pi}, and m_{4i} present (Fig. 36B). Macrosetae m_{2i2}, m_{4p}, and m_5 sometimes present; 2 additional macrosetae sometimes present on either side of m_{4p}. Th. 3 macrosetae a_1, a_2, a_3, a_4, a_{4i}, a_5, a_{5e}, a_6, a_{6i}, m_5, m_{5i}, m_6, m_{6e}, and m_7 present (Fig. 36C); most posterior (series P) macrosetae present, p_4 present or absent. Both thoracic segments with moderate number of supplemental macrosetae on zone Pm (Fig. 36B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 22 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length (Fig. 16D). Unguiculus acuminate with small serrations on internal edge.

Abdomen. Abdominal chaetotaxy reduced and slightly variable. Abd. 1 with 4 macrosetae: a_5, m_2, m_3, and m_4 (Fig. 36D). Abd. 2 with 5 macrosetae: a_2, m_3, m_{3e}, m_{3ep}, and m_5 (Fig. 36E); a_3 usually absent. Abd. 3 with 3 macrosetae: m_3, pm_6, and p_6 (Fig. 36F); a_3 and am_6 usually absent. Abd. 4 with 6 inner macrosetae (Fig. 36G).

Remarks. *Entomobrya unifasciata* sp. n. can be diagnosed by the presence of only three dark transverse bands (no band across the posterior margin of Th. 2), presence of head mesoseta An'_0, 4 macrosetae on Ab. 1, and 3 eye patch setae (see Table 2 for additional diagnostic characters). This species is part of the *E. ligata* complex (see remarks for *E. ligata*) and is very similar to *E. ligata* and *E. neotenica* sp. n., but can be separated by characters outlined in Table 6. Though identical in chaetotaxy, molecular evidence (Katz et al. 2015) and the absence of a dark transverse band on the posterior margin of Th. 2 separate this species from *E. ligata*.

Distribution. Endemic to North America (Suppl. material 2: N). Many records of *E. ligata*, especially those collected from the Smokey Mountain region west to the Mississippi River, are likely to be *E. unifasciata* sp. n.

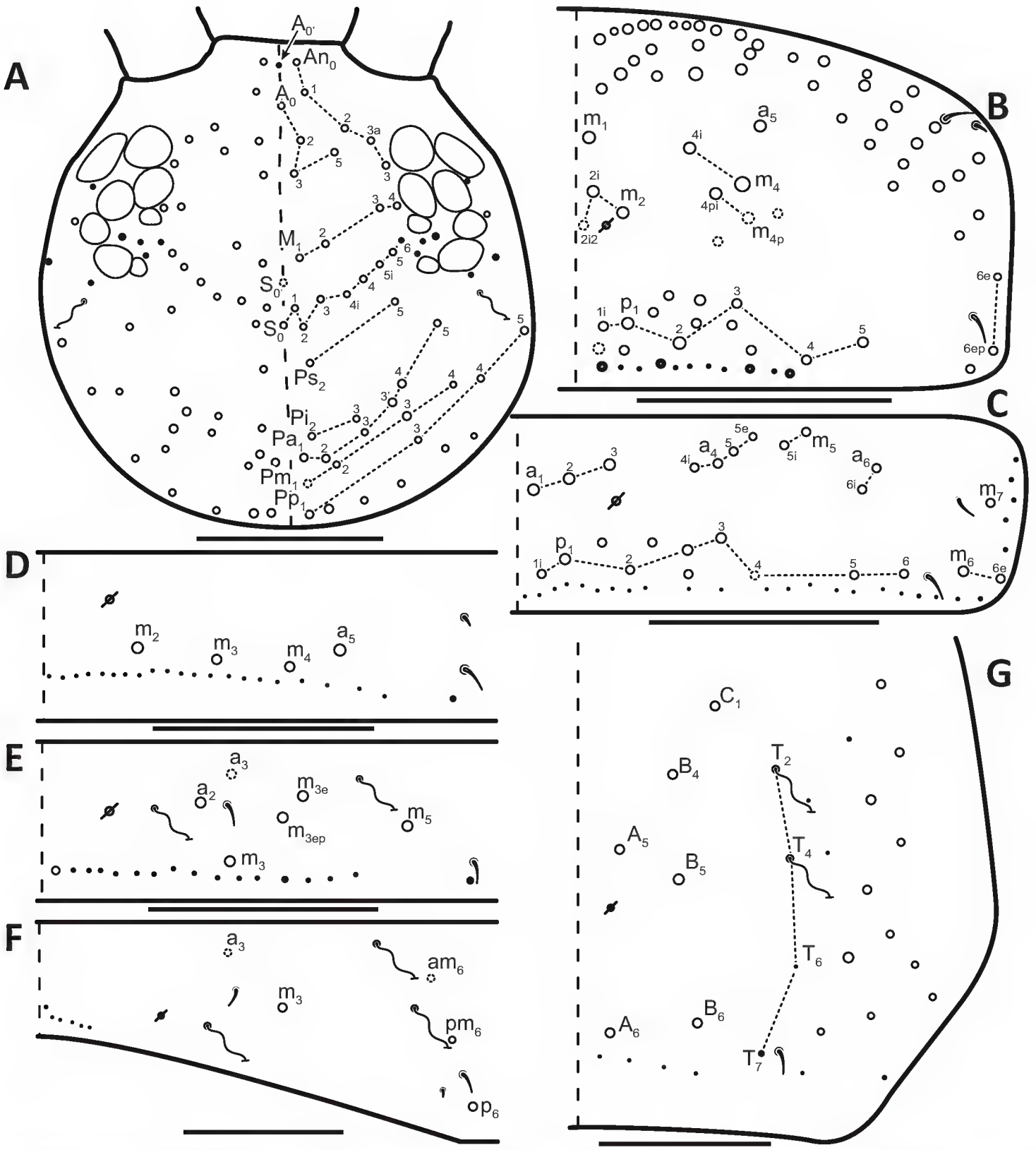


Figure 36. Dorsal chaetotaxy of *Entomobrya unifasciata* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

Entomobrya unostrigata Stach, 1930

Figs 2, 3C, 37, 38, 39

Description. *Body shape and color pattern*. Body relatively robust and cylindrical. Color form largely monomorphic (Fig. 37): white, pale green or yellow background with purple or black pigment forming a thin medial longitudinal stripe from anterior margin of Th. 2 to posterior margin of Abd. 5; band ostensibly thicker on Th. 3 through Abd. 3. Patches of pigment forming a lateral line along margins of Th. 2

Figure 37. Color patterns of *Entomobrya unostrigata*. Lateral (**A**) and dorsal (**B**) views of specimen collected from Chittenden Co., VT.

through Abd. 5, with variable spots of pigment present throughout. Abd. 5 and Abd. 6 usually lack dark pigment. Legs and furcula white and/or with light purple pigment. Antennae with light purple pigment.

Head. Apical bulb of 4th antennal segment usually bilobed. Long differentiated smooth setae on ventral side of 1st antennal segment ≈3× short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2 seta or spine-like projections (Fig. 2). Lateral appendage of labial papilla E extending just past tip of papilla. Dorsal head chaetotaxy (Fig. 38A) with macrosetae An'_0, $A_{3a2,}$ A_{3a3}, A_6, M_{3i}, S'_0, S_6, Ps_3, Pi_1, Pa_3, and Pm_{1i} absent; S_1, Pi_2, and Pm_1 usually present. Eyes G and H enlarged and similar in size to eyes C-F. Eye patch with 5 setae.

Thorax. Thoracic chaetotaxy well-developed and relatively stable. Th. 2 macrosetae a_5, m_1, m_4, m_{4p}, and m_5 present (Fig. 38B); posterior macrosetae (series P) present. Th. 3 macrosetae a_{5e2}, a_{5e3}, m_4, m_{5p}, and a_7 absent (Fig. 38C); P_4 usually present. Both thoracic segments with many supplemental macrosetae on zone Pm (Figs 3C; 38B,C).

Legs. Trochanteral organ with triangular setal pattern and up to 21 setae.

Abdomen. Abdominal chaetotaxy highly developed and variable. Abd. 1 with 7-10 macrosetae (Fig. 38D). Abd. 2 macroseta a_2, a_3, m_3, m_{3ep}, m_{3e}, m_{3ea}, and m_5 present (Fig. 38E); m_{3ei} usually present. Abd. 3 macroseta a_1, m_3, am_6, pm_6, and p_6 present (Fig. 38F). Abd.4 with 10-16 macrosetae present interior to bothriotricha T_2 and T_4 (Fig. 38G). Anterior half of Abd. 4 usually with 1 or 2 conspicuous rows of macrosetae. Apical mucronal tooth enlarged.

Remarks. *Entomobrya unostrigata* can be easily diagnosed by color pattern, a thin medial longitudinal stripe from the anterior margin of Th. 2 to the posterior margin of Abd. 5, combined with the absence of macrosetae Th. 2 m_2 and Abd. 3 a_2 and the presence of macrosetae m_5 on Th. 2 and a_1 on Abd. 3 (see Table 2 for additional diagnostic

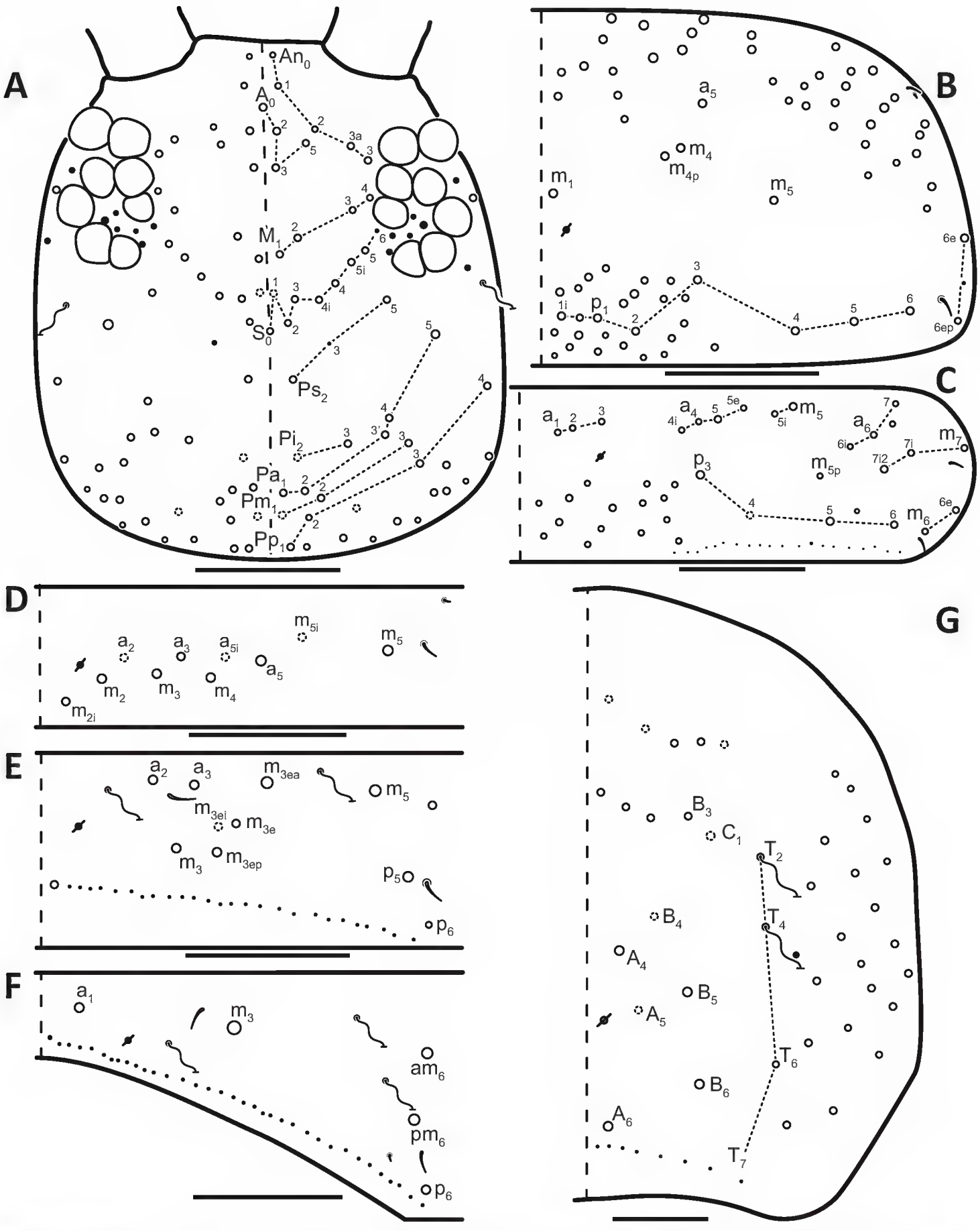


Figure 38. Dorsal chaetotaxy of *Entomobrya unostrigata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1st abdominal segment **E** 2nd abdominal segment **F** 3rd abdominal segment **G** 4th abdominal segment. Scale bars = 100 μm. See Figure 5 for symbol legend.

characters). This species exhibits atypical morphology for a Nearctic *Entomobrya*. Eyes G and H are greatly enlarged and similar in size to eyes C-F, an uncommon trait unobserved in other Nearctic *Entomobrya*. Additionally, macrosetae usually form 2 rows on the anterior half of Abd. 4; a pattern shared with many Palearctic *Entomobrya* and

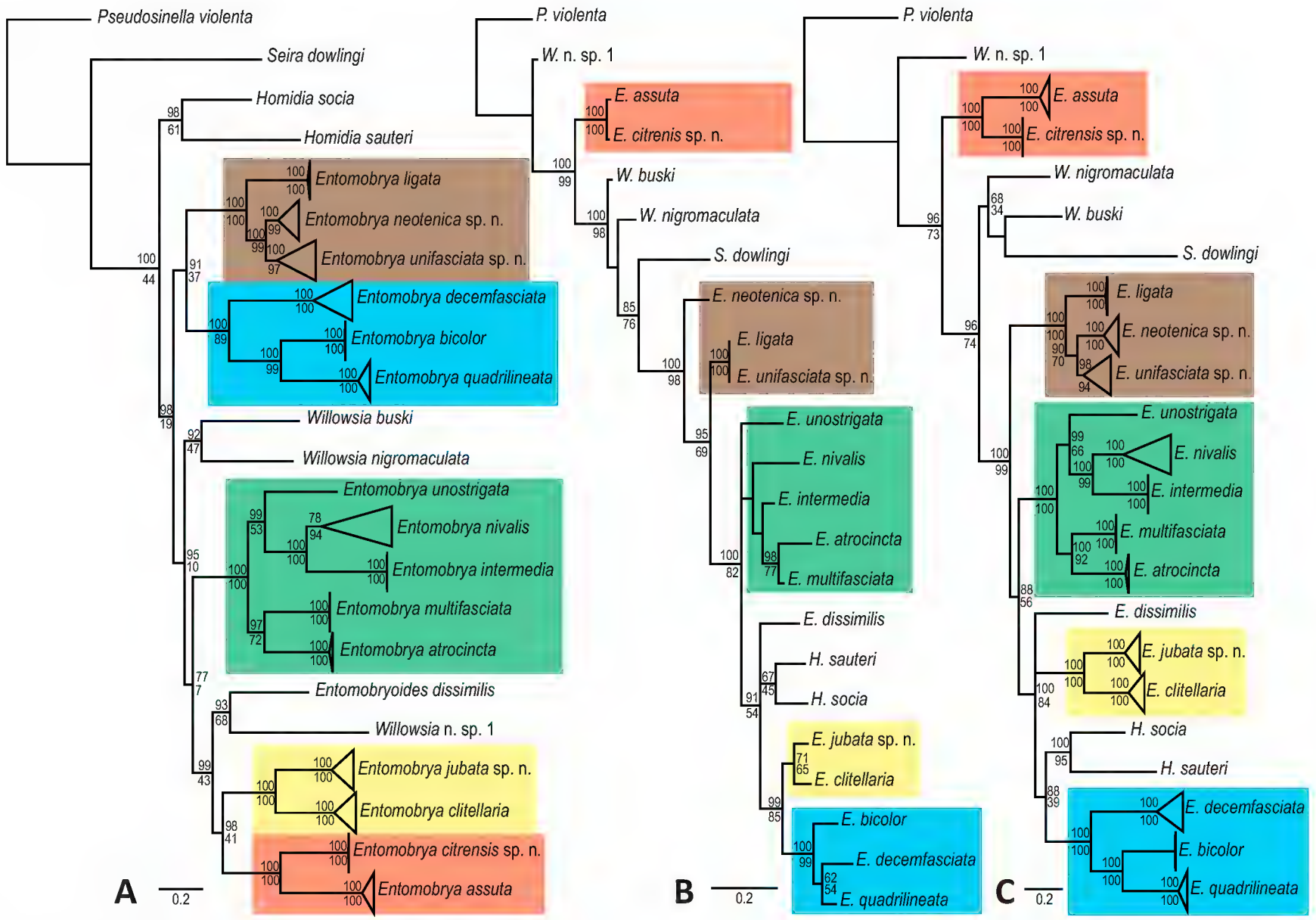


Figure 39. Bayesian 50% majority rules consensus trees with *Pseudosinella violenta* as the outgroup: **A** phylogeny resulting from the analysis of cytochrome *c* oxidase I DNA sequences (complete gene, 1539 bp) from Katz et al. (2015) **B** phylogeny resulting from the analysis of 179 morphological characters **C** combined phylogeny (COI and morphology). *Entomobrya* species groups are highlighted with colored boxes. Branches of representing taxa from the same species were collapsed into triangles (triangle length represents branch lengths between collapsed branches) for simplicity. Branch labels include posterior probabilities (above) and maximum likelihood bootstrap support (below). Scale bars represent 0.2 base substitutions.

also with members in the genus *Homidia*, but not with other *Entomobrya* treated here. *Entomobrya unostrigata* is a recently introduced species, now with a widespread Nearctic distribution (Christiansen and Bellinger 1998), which may explain its distinction from other North American *Entomobrya* species included in the present study. The *E. unostrigata* specimens observed for this study have only two large seta or spine-like projections on each labral papillae (Fig. 2). However, multiple variations of the labral papillae have been reported (Christiansen 1958b; Christiansen and Bellinger 1992; Christiansen and Bellinger 1998; Jordana 2012).

Distribution. Nearctic, Palearctic, and Australia (Suppl. material 2: O).

Material examined. USA: 2 on slides, Vermont, Chittenden Co., South Burlington, Vegetable garden on south side of Swift St. at intersection with Spear St. (44.4433,-73.1893), 3.viii.2003 (F. Soto-Adames); 1 on slide, Wisconsin, Dodge Co., Horicon Marsh National Wildlife Refuge, end of Dike Rd. (43.52736,-88.64381), 12.vi.2011, AK11-47.

Morphological phylogeny

Bayesian analysis of 179 morphological characters from 23 taxa produced a single consensus tree with high support (Fig. 39B). The most likely tree inferred by the maximum likelihood analysis is congruent with the Bayesian tree and bootstrap values were added to the Bayesian consensus tree (Fig. 39B). When *Pseudosinella violenta* is designated as the outgroup, the topology of the tree assumes a ladder-like evolutionary progression that is associated with an increase in the number of dorsal macrosetae. *Entomobrya assuta* and *E. citrensis* sp. n., the two species with the smallest number of macrosetae, form a monophyletic group at the base of the tree, whereas *Entomobrya bicolor*, *E. decemfasciata*, and *E. quadrilineata*, which are characterized by an abundance of dorsal macrosetae, form the most derived monophyletic group. This tree indicates that *Entomobrya* is paraphyletic, as the genera *Seira*, *Homidia*, *Entomobryoides*, and most representatives of *Willowsia* are interspersed among clades of *Entomobrya* species. The two species in the genus *Homidia* form a monophyletic group, but the genus *Willowsia* is not monophyletic. Other clades identified by the molecular COI analysis in Katz et al. (2015) (Fig. 39A) were not resolved as monophyletic groups based on morphological characters.

Combined phylogeny (COI and morphology)

Bayesian analysis based on combined morphology and COI (Katz et al. 2015) datasets yielded a highly supported tree (Fig. 39C). The most likely tree inferred by the maximum likelihood analysis is congruent with the Bayesian tree and bootstrap values were added to the Bayesian consensus tree (Fig. 39C). The addition of molecular characters resolves all monophyletic species groups/clades of *Entomobrya* observed in

the COI only tree (Fig. 39A). However, the deeper relationships among clades follow the progressive evolution towards an increased number of macrosetae supported by the morphological analysis. Both analyses (morphology only and combined) indicate that *Entomobrya* is paraphyletic.

Discussion

Diagnostic and phylogenetic utility of chaetotaxy

Detailed examination of the adult chaetotaxy of 15 species of North American *Entomobrya* suggests that the exclusive use of chaetotaxy for species diagnosis and as phylogenetic characters can potentially cause serious confusion. This study uncovered high levels of previously undocumented intraspecific variation and asymmetry of dorsal macrosetae (Fig. 13), and has made it clear that examination of many individuals is critical to properly identify variable chaetotaxy in order to choose appropriate characters for species delimitation. For example, Christiansen and Bellinger (1998) separate many species based on the chaetotaxy of Abd. 2 and Abd. 3, but in some species groups both regions contain significant intraspecific variation. In the Collembola of North America (Christiansen and Bellinger 1998), species within the *nivalis* group (*E. nivalis*, *E. atrocincta*, *E. multifasciata*, and *E. intermedia*) are separated by a combination of color pattern and by the presence or absence of four macrosetae: m_{3ep} on Abd. 2 and a_1, a_2, and a_3 on Abd. 3. This study shows that these characters must be used in combination to provide sufficient separation of these species due to overlapping intergrades of color forms and the presence macrosetae polymorphisms. Species in the *bicolor* group (*E. bicolor*, *E. decemfasciata*, and *E. quadrilineata*) present the most obvious example of the failure of chaetotaxy to provide an adequate means for species separation. Large numbers of macrosetae, extreme levels of inter-individual variation, and the common occurrence of asymmetries, cause significant overlap and obscure the homology of macrosetae otherwise considered important in species separation. The present study shows that color pattern is absolutely critical for the identification of species within this group.

These problems are cause for concern considering the important role of dorsal chaetotaxy in diagnosis and delimitation of species in the family Entomobryidae. Many studies are based on chaetotaxy as primary (or sole) evidence for species separation, following Szeptycki's (1979) nomenclatural system (Chen and Christiansen 1993; Jordana 2012). The homology of macrosetae as defined by Szeptycki (1979) assumes that a fixed number of macrosetae occur in strict, predefined positions rather than randomly distributed within a given area. Homologies are easier to determine between species in genera characterized by small numbers of macrosetae, and chaetotaxy likely provides accurate phylogenetic estimation in these groups. However, in groups such as *Entomobrya*, intraspecific variation, differences in setae arrangements, asymmetries, and large numbers of supplemental setae, render homology assessment a subjective and arbitrary process (Potapov and Kremenista 2008). Incorrect homology assignments obscure any useful phylogenetic information provided by chaetotaxy.

Post-embryonic studies that test Szeptycki's (1979) hypotheses have been successful in identifying and refining setae homologies in some pluri- and polychaetotic species and groups (Soto-Adames 2008; Zhang et al. 2011), but these studies are laborious and are only conclusive for species and/or groups examined. Information regarding the post-embryonic development of *Entomobrya* chaetotaxy is very limited and mainly concerned with Palearctic species (Szeptycki 1979). Explicit hypotheses concerning the underlying molecular mechanisms governing macrosetae development and position, to our knowledge, have only been tested in Diptera. These mechanisms are generally very complex and highly regulated processes, controlled by multiple genes that ensure macrosetae do occur in strictly defined locations (Simpson 1990; Heitzler et al. 1996; Leyns et al. 1996; Simpson et al. 1999; Furman and Bukharina 2008). However, it is uncertain (and unlikely) that Collembola macrosetae, and/or the genes that regulate them, are homologous to those of Diptera.

The phylogenies based on morphological and molecular data illustrate the effects that chaetotaxy has on phylogeny estimation. The trend of progressive evolution towards an increased number of macrosetae presented in the morphology and combined phylogenies is suspicious and may be driven by outgroup choice and character coding strategy. *Entomobrya assuta* and *Entomobrya citrensis* sp. n., sister species characterized by reduced chaetotaxy, are basal to all other *Entomobrya*, together with all scaled species included in the analysis (*Pseudosinella*, *Willowsia*, and *Seira*), which also have few macrosetae. This association is not reflected in the analysis of COI alone (Katz et al. 2015; Fig. 39A) and is likely due to the accumulation of characters (each seta in a multiplet was defined and scored as a separate character, see Suppl. material 1) present in polychaetotic species but absent in the *E. assuta* group and the outgroup. Evaluation of coding strategies for chaetotaxy, and the effect of outgroup choice is clearly needed to exact the maximum amount of phylogenetic information while minimizing homoplasy. Despite the conflict in branching pattern for deep nodes estimated by morphology and COI, the combined analysis retains the species composition and relationship of the five species groups identified by the COI analysis. These relationships are quite obvious and were proposed by Christiansen (1958b) before modern sequencing and dorsal chaetotaxy systems were available.

Phylogenetic relationships within Entomobryini and *Willowsia*

The result of the combined phylogenetic analysis (COI and morphology) of 15 species of North American *Entomobrya* in addition to 6 species representing 3 closely related genera (*Entomobryoides*, *Homidia* and *Willowsia*) and 2 outgroup species (*P. violenta* and *S. dowlingi*), generated a highly supported phylogeny of the North American Entomobryini and *Willowsia*. Several interesting results were observed concerning relationships among some currently recognized *Entomobrya* species. *Entomobrya nivalis*, *E. intermedia*, *E. multifasciata*, and *E. atrocincta* were resolved as a monophyletic clade; all are closely related but distinct species diagnosable using morphology and COI sequences. Overlapping intraspecific variation in color pattern and chaetotaxy

has caused many to consider these species synonymous (See Christiansen 1958b and Jordana 2012 for revision history). This study has, for the first time, combined substantial molecular (Katz et al. 2015) and morphological evidence in support of their separation. It is also worth noting that these were the only *Entomobrya* species included in the analysis with Holarctic distribution, all commonly reported throughout North America (Christiansen and Bellinger 1998) and Europe (Jordana 2012). The monophyly of the group and separation from species endemic to North America suggest a common origin in Europe or Palearctic region. It is possible that the group is either part of a shared, relictual Laurasian fauna or that the species were introduced from Europe in historical times. Evaluation of these two hypotheses will require molecular analysis of extensive samples of North American and European populations.

The resulting phylogeny also raises questions regarding the generic relationships within the tribe Entomobryini (here represented by the genera *Entomobrya*, *Entomobryoides* and *Homidia*) and *Willowsia*, a closely related genus. Research concerning the systematics of these groups is limited, and most phylogenetic studies have focused on suprageneric relationships by utilizing morphology, allozymes, and/or ribosomal markers that provided limited resolution and support of generic relationships (Lee and Park 1991; Lee et al. 1995; D'Haese 2002; Xiong et al. 2008). However, more recent work by Zhang et al. (2014b) indicated the non-monophyly of Entomobryini and Willowsiini based on molecular phylogeny. Zhang et al. (2014a) also questioned the monophyly of Willowsiini. Further analyses of S-chaetae (dorsal sensilla) dismissed Willowsiini as monophyletic (Zhang and Deharveng 2015).

This study supports Zhang et al.'s (2014a, 2014b) and Zhang and Deharveng's (2015) findings regarding the non-monophyly of Entomobryini and Willowsiini. Our phylogenetic analysis places both *Willowsia nigromaculata* (Lubbock) and *W. buski* (Lubbock) within the *Entomobrya* clade, further supporting the paraphyly of *Entomobrya*. This is not entirely unexpected considering the means by which these genera are differentiated. The genus *Entomobrya* is considered the most morphologically generalized group of Entomobryinae without distinct apomorphies while morphologically similar species with autapomorphic characters are separated into different genera (e.g., *Willowsia* with scales, *Entomobryoides* with smooth tibiotarsal setae and *Homidia* with dental spines). In light of the relationships inferred from the phylogeny, the classification of *Entomobrya*, along with some other genera within Entomobryidae, may need to be reevaluated in the future.

Results also indicate that the genus *Willowsia* may be polyphyletic. A new *Willowsia* species (*Willowsia* n. sp. 1) collected in Citrus Co., Florida, is resolved as a sister species to the *Entomobrya* clade, while *W. buski* and *W. nigromaculata* seem to have evolved from lineages within the *Entomobrya* clade. Considering their world-wide distribution, presumably spread by humans, and their similarity to Asian species (Zhang et al. 2011), *W. buski* and *W. nigromaculata* most likely were introduced into North America from Asia. However, *Willowsia* n. sp. 1 shares characteristics (i.e., scale type; see Zhang et al. 2011) with *W. mexicana* Zhang, Palacios-Vargas & Chen, the only other *Willowsia* species known to be endemic to North America (Zhang et al. 2007). *Willowsia* n. sp. 1's morphological similarity to *W. mexicana* may support an inde-

pendent origin of New World *Willowsia*. Further exploration by utilizing additional markers and more complete taxon-sampling among closely related genera is needed in order to establish appropriate generic relationships and classifications.

Acknowledgments

We would like to thank Ernest Bernard, R. Edward DeWalt, Christopher Dietrich, José Marcelino, and Steven Taylor for providing specimens, and the Systematics Discussion Group at the University of Illinois for their helpful suggestions and comments. Special thanks to Juraj Cech, Monique DuBray, and Benjamin Hottel for their valuable assistance in field collections. This work was supported by the Herbert H. Ross Memorial Fund and the School of Integrative Biology Enhancement Fund from the University of Illinois; a Society of Systematic Biologists (SSB) Graduate Student Research Award; the United States Department of Agriculture (Hatch program fund to R. G.); and the National Science Foundation (0956255 to F. N. S. A.). The collection of additional material was supported by the E.D.E.N. Azorean Habitats – Environmental Defense of Endangered Native Azorean Habitats (financed by The Luso-American Foundation and the Fundo Regional Ciencia e Tecnologia, Azores, PT, to José Marcelino); and the United States Department of Agriculture, Forest Service (Southern Illinois cave ecosystems: bioinventories, microbial communities & management plans grant to F. N. S. A.). We would also like to thank the Department of Entomology at the University of Illinois Urbana-Champaign and the Department of Biology at the University of Puerto Rico.

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Supplementary material 1

Morphology matrix

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: Nexus file optimized for Mesquite

Explanation note: Alignment nexus file for all morphological characters used for phylogentic analysis. This file is optimized for viewing in mesquite and includes all character state information.

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Supplementary material 2

Distribution maps

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: PDF file

Explanation note: Distribution maps: **A**, *Entomobrya assuta*; **B**, *Entomobrya atrocincta*; **C**, *Entomobrya bicolor*; **D**, *Entomobrya citrensis* sp. n.; **E**, *Entomobrya clitellaria*; **F**, *Entomobrya decemfasciata*; **G**, *Entomobrya intermedia*; **H**, *Entomobrya jubata* sp. n.; **I**, *Entomobrya ligata*; **J**, *Entomobrya multifasciata*; **K**, *Entomobrya neotenica* sp. n.; **L**, *Entomobrya nivalis*; **M**, *Entomobrya quadrilineata*; **N**, *Entomobrya unifasciata* sp. n.; **O**, *Entomobrya unostrigata*.

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Supplementary material 3

Photographs of type specimens

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: PDF file

Explanation note: Photographs of type specimens stored at the Illinois Natural History Survey, Champaign, IL. Lateral (**A**) and dorsal (**B**) views of a neo-paratype specimen of *Entomobrya quadrilineata* Bueker, 1939, Fountain Bluff, IL., V.15.1932, Ross & Mohr. Lateral view of a co- type specimen of *Entomobrya decemfasciata* Packard 1873 (**C**), no locality information. *Entomobrya ligata* Folsom 1924: Co-type specimens, Roorheesville?, NY 30Aug1923, M. J. Leonard (**D, E, F**); Co-type specimen, NY, July 1923, A. Wolf (**G**).

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